THE AETIOLOGY OF FOOD AND DRINK PREFERENCES, AND RELATIONSHIPS WITH ADIPOSITY

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UCL

DECLARATION

I, Andrea Dominica Smith, confirm that the work presented in this thesis is my own. Where information has been derived from other sources, I confirm that this has been indicated in the thesis.

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ABSTRACT

Food preferences are important drivers of actual food choice, determining micro- and macronutrient intake; and poor dietary quality increases the risk for nutrition-related disease. Greater liking for sweets, fats and snacks has sometimes been related to higher body fat in childhood, yet the relationship in adults remains unclear. Twin studies are a powerful design to understand the importance of nature and nurture in these behaviours. So far, twin research on food preferences has only used young paediatric or adult populations but the relative importance of genes and the environment in shaping these preferences in early adulthood, a period of increasing independence and autonomous food selection, remains unknown. In addition, drink preferences have received little attention, and there is a need to find out if 'unhealthy' preferences are modifiable.

This thesis uses data from TEDS, a large population-based cohort of 18-19 year old British twins, to assess the aetiology of food and drink preferences, and to investigate the association of food and drink preferences and adiposity, in late adolescence/early adulthood.

Study 1 describes the development of a self-reported food and drink preference questionnaire, confirming that food preferences cluster in six traditional categories: vegetables, fruits, meat/fish, dairy, snacks and starches.

Study 2 used the twin design to identify substantial genetic influences on preferences for six identified food categories and seven non-alcoholic drink types. In general, genetic effects were slightly higher for food than drink preferences, but the remaining inter-individual variation for all dietary preferences were influenced by non-shared environmental factors (any influences in the wider environment that make twins less similar despite their shared genes and home environment).

Study 3a established that cross-sectional associations between dietary preferences and BMI are limited in this age group; only higher liking for dairy foods and non-nutritive sweetened beverages was positively associated with higher adiposity in older adolescents. **Study 3b** used a BMI-discordant MZ twin design to show that when genetic and shared-environmental confounding is eliminated, food and drink preferences do not explain adiposity differences in genetically-matched individuals. This design allowed to rule-out genetic or shared environmental factors as contributors to BMI-discordance.

Lastly, **Study 4** developed and piloted a short three-arm randomized controlled trial comparing two sugar reduction strategies (gradual vs. immediate cessation) to assess the feasibility of sweetness preference modification in relation to hot beverages, i.e. hot tea. Intake of sugar in tea decreased substantially in both sugar reduction conditions, without a loss in overall liking of tea.

A better understanding of the aetiology of food and preferences, particularly identifying the importance of the wider environment as a salient shaper of both food and drink preferences, and their relationships with adiposity, has important implications for researchers, policy makers and clinicians. Establishing the feasibility of sweetness preference modification in beverages without loss of liking for the beverage is also important for public health initiatives, suggesting that such preference change is possible and likely sustainable over the long-term.

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Chapter 1.

Literature review on food and drink preferences

1.1 The importance of food and drink preferences for health and energy balance

Dietary energy intake is a vital contributor to the development of obesity and associated co-morbidities (Bulló et al. 2007). Food and drink preferences are key upstream determinants of actual intake (Drewnowski 1997; Domel et al. 1996; Domel et al. 1993; Lennernäs et al. 1997; Biloukha & Utermohlen 2001; Glanz et al. 1998; Kourouniotis et al. 2016), thus greater preference for foods and drinks that are higher in energy may contribute to the development of excess body weight

For this thesis, food and drink preferences are defined as increased liking for specific food and drink items, or food groups (not food choice or actual intake). In other words, a food and drink preference is the affective response (i.e. the pleasure and enjoyment) an individual experiences from tasting food or drink. Preference is thus not being used as a relative term, i.e. preferring one food type (or group) over another.

1.1.1 Healthcare and societal costs of poor nutrition

The Global Burden of Disease study estimated that in 2016, around 9.6% of the world's entire health burden and 18.8% of all deaths were attributable to sub-optimal dietary intake. Of the individual dietary risk factors, the largest proportion of disability-adjusted life-years (DALYs) were lost due to diets low in whole grains (2.6% of global DALYs), low in fruit (2.6%), and low in nuts and seeds (2.1%). For number of deaths, a diet low in whole grains (4.6%), low in fruit (4.3%), and high in sodium (4.2%) were the leading risk factors (GBD 2016 Risk Factors Collaborators et al. 2017).

Over the past few decades, economic development and demographic changes have led to shifts in the global diet, the so-called "nutrition transition" (Popkin 2006). The globalisation of food trade and marketing has fuelled the spread of these changes worldwide. Cheap and palatable food has never been easier and less time consuming to access in most middle- and high-income countries. This shift in nutrition habits has coincided with a decrease in physical activity and an increase in sedentary behaviour. Given that overweight and obesity arise from sustained energy imbalance - excess energy intake relative to energy expenditure – these changes have led to an 'obesogenic' environment; one that encourages the development of excess adiposity. But despite the fact that we are all presented with an 'obesogenic' environment, there is still substantial variation in adiposity at a population level, suggesting that individuals differ in their susceptibility to these environmental factors (Flegal & Troiano 2000). Researchers have therefore been interested in understanding the basis of variation in vulnerability to the 'obesogenic' environment. Variation in preferences for energy-dense foods and drinks may offer one explanation.

Studies have shown that food and drink preferences are drivers of dietary choices and predict food intake (Drewnowski & Hann 1999). However, findings linking specific food and drink preferences to adiposity outcomes remain inconsistent and warrant further investigation (Donaldson et al. 2009; Low et al. 2016; Cox et al. 2016). Improved understanding of the origin of differences in food and drink preferences is also important, as this will inform the development of preference modification programs – e.g. behavioural interventions to increase individuals' preferences for nutritious food (e.g. vegetables) or decrease liking for energy-dense and micronutrient-poor food (e.g. sugary treats). Experimental studies that explore the feasibility of modifying preferences offer additional insight into the potency of environmental factors in shaping food preferences, and potential strategies that might be implemented on a wider scale to effect change.

1.1.2 Measuring food and drink preferences

A wide range of measures can be used to quantify liking for food and drinks; but each varies in reliability and sensitivity. Food and drink preferences can be measured using different strategies: (i) quantifying an individual's *liking* for certain flavours, sensory attributes, or actual food or drink types; and (ii) ascertaining an individual's *relative* food or drink preference by asking them to choose one food or drink over another (so-called 'forced choice'), or to rank them in order of preference.

Before details of various food and drink preference measures are discussed, it is important to distinguish between 'liking' and 'wanting'; two distinct traits that are both implicated in food and drink preferences (Mela 2006). 'Liking' of food and drinks strictly refers to the affective responses (hedonic pleasure and enjoyment) related to consumption of food or drink. In comparison, 'wanting' captures the incentive salience of the food or drink; i.e. the *motivation* to consume something, which is triggered by a food cue that stimulates a neural reward representation that makes it attractive

(Finlayson et al. 2007). These two processes are distinct – for example, one can like a food without wanting it. Both are important in influencing food and drink intake.

There are two main ways to measure food and drink preferences, laboratory-based tasting or psychometric questionnaire–based assessments of food and drink preferences. Laboratory-based measures of food and drink preferences offer more precise measurements, allowing for the observation of people's actual choice, but these measures can be influenced by extraneous factors at play on the day and offer only a single 'snap shot'. They are also expensive, time-consuming and can be onerous for the participants and researchers. The observed ratings can be recorded on continuous scales (e.g. a visual analogue scale (VAS), a labelled magnitude scale, or a Likert scale), or participants are asked to indicate their relative preference for one item over another.

Psychometric (questionnaire) measures provide a good alternative measurement method; participants are asked to rate their liking for individual food and drink items using a continuous response scale, such as a Likert scale, or to rank their preferences for different foods. Although questionnaires may lose the precision of laboratory-based tests (e.g. relying on a participant remembering how much a particular food is liked, in the absence of that food), arguably they capture the 'general rule' rather than a single snap shot. And people tend to know and remember which foods they like and dislike, without requiring a taste of that particular food in order to report on it. Questionnaires also enable large-scale data collection so that population-based estimates can be derived, and they are cheaper, and are less onerous for participants to complete because they do not require them to travel to a laboratory for testing. Questionnaire measures can also be validated against behavioural measures in the laboratory, especially for parent-reported measures of their children's food preferences (Pliner & Pelchat 1986). Mean liking scores for each individual food type are calculated, and these can be aggregated into broader food groups based on their nutritional content (e.g. macronutrients), or traditional food categories (e.g. vegetables, fruit, meat, dairy), or aggregated using data driven methods such as Factor Analysis or Principal Components Analysis.

There are limitations with all types of measures of food preferences. One is the potential for social desirability bias, the phenomenon whereby participants report responses that they perceive as more desirable to avoid criticism or shame, and to gain approval (Grimm & Grimm 2010). Participants may therefore report higher liking for 'healthier' foods and drinks and lower liking for 'unhealthy' foods and drinks, than actual liking. If misreporting is higher among higher adiposity individuals, this bias can adversely affect results. This effect may be more prevalent in females than males, as women tend to be more influenced by social desirability bias than men, and experience more pressure and guilt surrounding their eating behaviours and body shape (Hebert et al. 1995; Wardle & Beales 1986; Hebert et al. 1997). Laboratory-based measures of liking may introduce more opportunity for social desirability bias than questionnaire-based measures that are reported privately at home.

1.1.2.1 Validation studies of food preference questionnaires

Given the heterogeneity of food preference measures, in addition to the notoriously challenging nature of dietary assessment, validation studies to ensure dependability of the used method remain crucial. Several efforts have been made to establish the strength of different food preference measures as determinants of food intake. In the following section, key studies that have attempted to validate food preferences measures, and the findings relating to the strength of food preferences measures as predictors of actual food intake, are summarised.

Overall, eight studies have attempted to validate food preference measures in children, and three studies in adult samples. Comparability between these studies is challenging due the substantial heterogeneity in food preference and dietary intake measures, as well as specific sample demographics; yet a broad understanding of the validity of these measures emerges.

Of the eight studies in children, seven have found low to moderate associations between food preference measures and measures of food intake, or food consumption frequency. In one of the first validation studies, Birch (1979) showed that food preference scores predicted subsequent food intake in 3–4-year-old children (r=0.80), thus demonstrating predictive validity for food-preference ranking measure (Birch 1979). In this small study, food preference data and consumption measures were collected directly from children during break time over the course of 4 days. Children were offered eight different open-faced sandwiches and were instructed to rank these in order from most to least preferred. Despite the high correlation coefficient seen between preference and food intake in this study, it is important to take into account that it was undertaken in a very small sample of 17 children, and focused entirely on food preferences from a very restricted spectrum of food items (sandwiches).

A different approach to estimate relative validity of a food preference measure is to estimate the agreement between child-rated preference scores with parent-reported food consumption frequency of their child. One such example is the study by

Vereecken et al (2010) in a group of pre-schoolers (n=135; mean age: 5.2 years). Vereecken et al used an interactive animated food preference measurement tool tailored to the age of the children. Participating children were required to rate their liking for 10 fruit and 10 common vegetables using a 3-point facial hedonic scale displayed on an iPad (Vereecken et al. 2005). Predictive validity was statistically significant but low for child-reported fruit preferences and parent-reported fruit intake (r=0.19), and moderate for child-rated vegetable preference and parent-reported vegetable intake (r=0.25).

More commonly, validation studies compare food preference ratings and food intake data obtained directly from the same individual. In the US-based ‘Gimme 5 Study’, correlations between fruit and vegetable preference (10 fruit items & 10 vegetable items) and snack preference (8 items) scores, rated on a 3-point Likert scale, with fruit and vegetable intake, were significant but low (r=0.26 for fruit and vegetables; r=0.16 for snacks). Dietary intake of fruit, vegetables and snacks was assessed via 7-day diet diaries in this sample of school-children (n=1398) aged 7-11 (mean age: 8.7 years) (Resnicow et al. 1997). Two further studies have suggested that the strength of the relationship between food preference and food intake tends to increase across childhood. In a study of 86 Australian preschool children (3-6 years), the percent agreement between self-reported food preferences (10 forced-choice photo pairs rated on an iPad) and actual food choice (the corresponding 10 forced-choice food pairs) was, on average, high at 73% (Wiseman et al. 2017). The percent of agreement increased significantly with age, rising to 78% in the 5-year-old children of that sample. In line with these findings, a study of 197 primary school girls demonstrated that preference ratings and food intake significantly correlated, and that correlation coefficients increased from r=0.33 for 5-year olds to r=0.45 for 11-year olds (Rollins et al. 2011). Liking for 10 common high sugar, high fat snack foods was measured on a 3-point facial hedonic scale. Overall, data from this study suggested that snack food preference scores became significantly stronger predictors of snack food calorie intake (measured in an ad-libitum meal) with age, despite interindividual variability of the relationship remaining high at each time-point.

Another study relied on a forced-choice measure of food preferences to quantify the relationship between child-reported preferences and dietary intake habits in 1696 children (6-9 years). This study of primary-school age children from eight European countries found no evidence in support of a significant relationship between greater liking of higher fat and more intensely sweet versions of foods and their consumption frequency (Lanfer et al. 2012). Forced choice pairs of high- vs low fat foods (e.g. crackers) and drinks (e.g. apple juice) were used to measure taste preferences while parental report questionnaires were used to approximate children's consumption frequency of fatty and sweet foods. However, when considering these findings, it is important to take into account that this study did not actually measure food preferences, but rather focused on taste intensity preferences for sweet and fatty foods.

To consider relationships between a wider range of dietary preference measures with dietary intake, two studies made use of a comprehensive multi-item food preference questionnaire. Domel et al reported that fruit and vegetable preferences indices measured on a 31-item self-report questionnaire were consistent predictors of fruit and vegetable intake, especially vegetable consumption, among fourth and fifth grade children (n=392). However, on the whole, fruit and vegetable preferences accounted for only small proportions of the variance of fruit and vegetable consumption (as recorded in 7-day food diaries), implicating other psychosocial factors as important influences on actual fruit and vegetable intake (Domel et al. 1996). In the other study, food liking was measured using a 5-point Likert scale for 77 common food items in a group of 125 girls polish school girls (13-15 years). A calibrated food frequency questionnaire was used to measure habitual dietary intake for the food items under consideration. At the single food item level, food preference scores and food frequency measures correlated significantly for 60/77 items. However, when food preference item scores were categorised into six main food preference groups (cereal products, vegetables, fruit, vegetables, meat/fish/egg, and sweets), associations with consumption frequency were moderate and significant (r~0.6-0.85) for all but the ‘sweets’ category (Czarnocińska et al. 2009).

Fewer studies have quantified the validity of food preference measures used to study the dietary preferences of adults, perhaps reflecting the belief that this relationship is stronger and less confounded by lifestyle and dieting behaviours in children. All three validation studies in adults’ studies relied on self-reported food preferences.

There was only one study which related food preference scores to researcher-observed food consumption. In 1963, Pilgrim and Kamen reported that in a communal army canteen, 31% of researcher observed variability of food consumption habits were explained by self-reported food preference ratings (Pilgrim & Kamen 1963). Preference ratings in this study had been obtained from 2000 army men, asking them to rate their liking for 72 food items on a 9-point Likert scale. The two other studies assessed concurrent validity of food preference measures by comparing food preference questionnaire data with food frequency questionnaires. In one study of undergraduate female students (n=87), food preference scores from a 98-item food questionnaire were found to significantly predict dietary intakes (n=87) (Drewnowski et al. 1999). Associations between virtually all item pairs of reported food preferences and 3-day food recall data were significant (mean r=0.40).

Concurrent validity of a food preference questionnaire and self-reported food frequency scores was slightly lower in a study of 150 Canadian men and women (Carbonneau et al. 2017). In this study, food preference scores were moderately correlated to food consumption frequency, ranging from r=0.19 for cookies to r=0.39 for poutine. Completion of this 50-item food preference questionnaire required participants to rate their liking for a variety of sweet and savoury food items on a 9-point Likert scale. Frequency of food intake was estimated with a web-based food frequency questionnaire validated for use in a French-speaking Canadian population.

The studies above demonstrate that the relationship between food preference and food intake differs in relation to numerous important factors, including sample demographics, the type of food preference measure, and the number of contextual influences taken into account. This reiterates that food preferences should not directly be interpreted as predictors of food intake in a specific situation; food liking only explains a part of the variation in food choices and eating behaviours. Nevertheless, the relationship between food preference measures and food intake (assessed using experimental or diet diary data) appears slightly stronger in children and teenagers, compared to the relationship reported in studies using adult subjects. A key challenge in the field of dietary preference research remains the absence of a gold standard. Irrespective of this, the wide range of food and drink preference measurement methods provide useful tools to quantify these traits in observational or experimental studies facilitating investigation into the underlying factors contributing to inter-individual variation in these traits.“

1.1.3 Associations of food and drink preferences and weight or adiposity

The following section reviews the existing literature that has investigated the relationship between food and drink *preferences* and adiposity.

1.1.3.1 Summary of the literature on the associations of food and drink preferences and individual weight or adiposity

Fourteen studies were identified that reported associations between food and drink preferences and adiposity. Overall 8/14 of the studies found significant positive associations between psychometric or laboratory-based measures of food item or food group preference ratings, and BMI or skinfold fatness percentages. The studies are reviewed in detail below, and summarised in **Appendix A1.** Before a detailed review of the literature is presented, two important points need to be highlighted.

Comparing findings across the 14 studies is difficult due to substantial heterogeneity in measurement of both preferences and adiposity, as well as differences in sample characteristics. Eight of the 14 studies include observations from toddlers and children (Laureati et al. 2015; Hill et al. 2009; Ricketts 1997; Fisher & Birch 1995; Lanfer et al. 2012; Diehl 1999; Fletcher et al. 2017; Lakkakula et al. 2008); the other six studies involve predominantly adults (Conner & Booth 1988; Davis et al. 2007; Matsushita et al. 2009; Nakamura et al. 2001; Duffy et al. 2007; Duffy et al. 2009). A fundamental issue when examining food and drink preferences is the substantial heterogeneity of measurement methods employed to quantify these traits. Food preference questionnaires were the most commonly used method to collect preference scores for lists of food and drink items; this method was used by ten of the studies (Davis et al. 2007; Hill et al. 2009; Matsushita et al. 2009; Nakamura et al. 2001; Duffy et al. 2007; Duffy et al. 2009; Diehl 1999; Conner & Booth 1988; Laureati et al. 2015; Fletcher et al. 2017; Lakkakula et al. 2008). Preference ratings on these questionnaires were collected on Likert scales with response options ranging from 3-9 points. Two studies collected item preference ratings from questionnaires using general labelled magnitude scales (gLMS) that rely on cross-modality-matching - whereby individuals are required to indicate their preference relative to an unrelated 'absolute comparator' (in this case 'strongest imaginable like' to 'strongest imaginable dislike'). However, there is no 'gold standard' food preference questionnaire, with substantial variability in the food items included (and the groups that the food items are aggregated into subsequently), as well as the preference rating options.

Laboratory-based testing of food and drink preferences, whereby individuals taste food or drink samples and then record a liking score for the items on a visual scale, were used by 5/12 of the studies (Laureati et al. 2015; Ricketts 1997; Fisher & Birch 1995; Lanfer et al. 2012) (one study used both a questionnaire and a laboratory-based sensory test (Conner & Booth 1988)). Three of the identified studies used the method of 'forced choice testing' to measure food preferences in relation to BMI. One of these studies used a forced-choice questionnaire with pairs of food and drink names (e.g. asking participants to select: 'cheeseboard OR cake'?) (Conner & Booth 1988), and the other two studies asked participants to choose their preferred foods between actual food and drink samples presented by a researcher (Lanfer et al. 2012; Ricketts 1997).

In adults, the sample sizes for these studies have ranged from n=88 (Duffy et al. 2009) to n=29103 (Matsushita et al. 2009); the latter being the only population-based study investigating food preferences in relation to adiposity. However, in general sample sizes have been small and except for the Matsushita et al study, all adult samples comprised <1000 participants. Studies undertaken in children range in size from n=18 in a sample of three to five year-old toddlers (Fisher & Birch 1995), to n=1696 in the multi-national 'IDEFICS' study, investigating the dietary habits and preferences of six to nine year olds (Lanfer et al. 2012).

Almost all (12/14 studies) studies of the relationship between food and drink preferences and adiposity were cross-sectional (Conner & Booth 1988; Davis et al. 2007; Nakamura et al. 2001; Duffy et al. 2007; Duffy et al. 2009; Laureati et al. 2015; Hill 2002; Ricketts 1997; Fisher & Birch 1995; Lanfer et al. 2012; Diehl 1999), making it impossible to draw conclusions about the direction of causation. The following section reviews the results from cross-sectional studies in adults, followed by an overview of the cross-sectional studies undertaken in children and adolescents. Lastly, findings from the two prospective studies on the relationship between food and drink preferences and adiposity are discussed.

1.1.3.2 Cross-sectional adult studies

Five of six cross-sectional studies in adults reported significant associations between preferences for fatty or high sugar foods and BMI. Davis et al (2007) asked 151 adult women to report their liking for 72 common food items (on a 9-point Likert scale ranging from 'dislike extremely' to 'like extremely'), which were grouped into 'high fat' and 'high sugar' categories. Food preference scores for 'high fat' but not 'high sugar' foods were associated with higher BMI scores (Davis et al. 2007). However, ceiling effects may have limited variation in the data and the ability of the study to find an effect (i.e. most participants had high liking scores for high sugar foods).

Three of the other studies found significantly higher preference for fatty foods among participants with higher BMIs (Duffy et al. 2007; Duffy et al. 2009; Nakamura et al. 2001). The food preference questionnaires used in the two US-based studies by Duffy et al recorded preference scores for 19 and 23 items respectively, using hedonic labelled magnitude scales ('strongest imaginable like' to 'strongest imaginable dislike'). The items on the questionnaires had been chosen to measure liking for foods high in fat (e.g. mayonnaise, cheese), sweet foods high in fat (e.g. cookies, cakes), fibre-rich foods (asparagus, blueberries, oatmeal, whole wheat bread), salty foods (e.g. sausages, bacon) and bitter foods or drinks (e.g. broccoli, coffee). In the first study, higher liking for fat and fibre-rich foods was positively associated with BMI and waist circumference in a sample of healthy male adults (n=422; mean age: 46 years). In the later study, higher liking for both fatty foods and fibre-rich foods was significantly associated with higher BMI in a small sample consisting only of females (n=88; 25-55 years). On first consideration, higher liking of high-fibre foods being associated with higher BMI may appear unusual however it is possible that the observed positive association is the reflection of higher liking for food in general in individuals with a higher BMI.

A larger study (n=892) undertaken in Japan by Nakamura et al relied on a simpler measurement methodology, asking the participants to report their preference for "fat rich foods" (which in the Japanese language has a slightly negative connotation), "fat rich meats" or "butter/lard" on a 4-point Likert scale ('yes, very much' to 'no'). Positive associations between the higher liking of fat-rich foods were significant for BMI, waist-to-hip ratio, subscapular skin thickness and abdominal skin thickness in males, but only significant for BMI and subscapular skin thickness in females. A different study focusing on the measurement of sweet foods, predominantly in UK adults (n=344; age range: 6 – 65 years), found no significant associations between BMI and preferences for sweet foods measured using forced choice questionnaires, a laboratory-based sweetness sensitivity test and a food frequency questionnaire (Conner & Booth 1988).

1.1.3.3 Cross-sectional studies of children and adolescents

Three out of seven cross-sectional studies in children found significant associations between sweet or fatty food preferences and adiposity. In a large multi-country study in primary-school aged children (n=1696), Lanfer et al (2012) reported significantly higher odds for overweight and obesity in children who reported higher liking for sweeter and fattier snack foods in a forced-choice testing scenario. In this Europe-wide study, the children were invited into laboratories and instructed to taste and rank a low and high fat version of a simple cracker, and a low and high sugar version of apple juice. Another study found that liking for a higher-fat snack (cookie, cake or brownie) compared to the liking of a lower-fat equivalent was also positively associated with adiposity in a small study (n=88) of 9-12 year-old American children (Ricketts 1997). In a very small study (n=18) of preschool American children, liking for higher fat items (e.g. peanut butter, chicken nuggets, cheese and margarine) was significantly associated with triceps skinfold thickness, but not weight-for-stature or subscapular skinfold thickness. However, the null associations were moderate in size (e.g. correlation coefficient r=0.3) and in the expected direction; non-significant estimates therefore probably reflected insufficient power to detect significant findings due to the very small sample (Fisher & Birch 1995). Only one study found a significant association between liking for fruits and vegetables and adiposity. In this study of US school children (n=314; 9-11 years), a 5.5x-fold increase for being at risk of overweight, or being overweight, was seen in children who reported low liking for fruit and vegetables (Lakkakula et al. 2008). This finding was based on preference scores for 17 fruit items and 21 vegetables which were rated on a 3-point Likert scale. However, preference scores for fruits only or for vegetables only, were not independently associated with adiposity status.

Three other studies have failed to observe significant associations between food and drink preferences and adiposity in children. In a sample of British primary-school aged children (n=366), overweight was not related to liking for fatty/sugary foods, vegetables or fruits (Hill et al. 2009), reported by parents on a preference questionnaire of 50 food items. In keeping with this study, in a sample of six to nine year-old Italian school children, liking for four fruits (e.g. apple, pear) and vegetables (e.g. fennel, broccoli) was investigated in relation to BMI (Laureati et al. 2015). Children tasted raw samples and rated their liking for each fruit and vegetable on a 7-point hedonic scale, but none of the preference scores were significantly associated with BMI, which may be attributable to the limited range of food preferences surveyed.

In the only study which included older children and young teenagers in Germany (n=1233; 10-14 y), liking of 114 food items and 14 drink items were measured by a food preference questionnaire and related to BMI z-scores, calculated from self-reported heights and weights (Diehl 1999). Preference ratings were recorded on a 5-point facial hedonic scale, instructing participants to circle 1/5 depicted faces, showing varying degrees of a smile, neutral expression, or frowning face that correspond to their personal preference for the listed items. For males, the findings were somewhat surprising; preference scores for 6/114 foods that were high in fat and/or sugar (sweet pancakes, cake, cookies, chocolate, chocolate bars and boiled sweets) were *negatively* associated with BMI z-scores. In contrast, positive associations between liking of only 4/114 items (semolina pudding, muesli, grapefruit and hazelnuts) and BMI z-scores were observed for the female participants. These rather contradictory results may perhaps arise from the fact that youngsters, and particularly girls, in this age group are particularly susceptible to bias. For example, the teenage girls in this sample may have been dieting, and the foods associated negatively with adiposity (chocolate, cookies and other treat foods) are the types of food a girl on a diet would actively avoid, and thus rate negatively.

1.1.3.4 Prospective study of adults

Only one study has examined the prospective association between food and drink preferences and weight gain in adults. The Japan Public Health Centre-based Prospective Study (Matsushita et al. 2009), a large study (n=29103; baseline age range: 40 – 59 y) of Japanese adults, measured food preferences using two questions: ‘Do you like rich and heavy food?’, and, ‘Do you like sweet foods?’ on a 4-point Likert scale. Higher preference for rich and heavy foods was significantly associated with weight gain in both men and women over a follow-up period of 10 years, whilst the preference for a sweet taste was a significant predictor of weight gain in women only.

1.1.3.5 Prospective study of children

There was also only one study in children that investigated the longitudinal association between food and drink preferences with adiposity measures. The Gateshead Millennium Study recorded parent-reported food preferences when their children were on average 30 months old (n=456). The food preference questionnaire in this study recorded preference ratings on a 5-point Likert scale for 11 different vegetables and seven fruits. When the children were seven to eight years of age (n=346), the number of fruits and vegetables liked at 30 months was not associated with researcher measured BMI, bioelectrical impendence fat and skinfold Z-scores (Fletcher et al. 2017).

1.1.4 Summary of the findings, strengths and limitations of the current literature on the association between food and drink preferences, and measures of adiposity

In total, 14 studies were identified that investigated the relationship between food and drink preferences and measures of adiposity. There was only one study in older children/young adolescents. Overall, there is evidence to suggest that preference for foods high in sugar or fat are positively associated with higher adiposity in adults as 5/6 studies reported significant associations between greater liking for these food and drink types and greater adiposity (usually indexed using BMI). The findings for children were less clear with only 3/8 studies reporting positive associations between greater liking for high sugar or fat foods and higher adiposity and one study reporting a negative association (for boys only).

This review has highlighted both the diversity of the foods and drinks that have been measured, as well as the varying indices of adiposity. The heterogeneity of these studies makes it challenging to draw any definite conclusions on the association of food and drink preferences and adiposity. Many existing studies have not fully explored the broad spectrum of commonly available food; e.g. fruit and vegetables, whole grain products or dairy foods. Perhaps understandably, the main focus has been on measurement of preferences for fatty or sweet foods which are most commonly implicated in excess weight gain.

For example, one of the first studies to explore the effect of fat on hedonic responsiveness and food liking was undertaken by Drewnowski and Greenwood (1983). In this study it was demonstrated that responsiveness to sweetened dairy increased significantly ($p<.01$) upon increasing the absolute fat content of milk- and cream-based samples from 2% fat (skim milk) to 38% fat (heavy cream) in healthy weight undergraduate students (n=16). Moreover, preference ratings (on a 9-point Likert scale) rose with an increase in fat, and did not plateau, potentially implicating fat in foods as a sensory aspect of food possibly driving overconsumption (Drewnowski & Greenwood 1983). This point has been explored elsewhere when Mela and Sachetti (1991) demonstrated a moderately positive correlation between overall fat preference and percent body fat ($r=0.46$, $p<.01$), supporting the idea that individuals with overweight or obesity perceive greater liking for high-fat foods (Mela & Sacchetti 1991). Participants were asked to taste 10 different foods with up to five varying levels of fat, and to indicate the preferred mean level at which the food item was most palatable. In addition, this study on dietary fat preference also revealed that liking for fats is context specific, and that preference for a higher fat version of one type of food (e.g. cookie) does not extend to liking for other types of foods (e.g. salad dressing).

More recently in a large population-based study in France (n= 24,776), it was also shown that greater liking for fat in foods was prospectively linked to a significantly greater risk of obesity for both men (HR of 2.39 (95% CI: 1.39, 4.11), and women (HR of = 2.02 (95% CI: 1.51, 2.71)) for individuals in the highest quartile of fat preference compared for individuals in the lowest quartile. The participants in this large population-based cohort were drawn from the NutriNet-Santé study for which researchers developed a comprehensive questionnaire of food and taste preferences, the PrefQuest questionnaire. The PrefQuest includes composite subscales on fat preferences consisting of 9-point hedonic Likert scale ratings for ‘fatty-salty’ (31 items) and ‘fatty-sweet’ foods (20 items). It further includes various questions on the frequency of a several dietary behaviours (e.g. addition of high-fat condiments to food) to capture a broad overview of the multidimensional nature of a taste preferences such as fat (Lampuré et al. 2014). Collectively, these studies provide some evidence that greater liking for fat in foods may indeed be linked to greater risk of adiposity.

However, vegetables and fruits are low in energy density and so it is possible that greater liking for these foods offers protection against excess adiposity. Only four studies assessed preferences for fruits and vegetables and adiposity, and three reported null findings (Hill et al. 2009; Laureati et al. 2015; Fletcher et al. 2017). However, greater preference for fruits and vegetables would only protect against overweight if higher preference leads to higher intake (Mytton et al. 2014); and these studies were in primary school children who have far less control over their actual intake than adolescents or adults. It might be possible to observe significant relationships between food and drink preferences and adiposity in older samples who have more autonomy over their food choices.

Notably there is also a real gap in the literature on preferences for different types of drinks in relation to adiposity. Of the 14 studies, only three assessed preferences for beverage types, and only one presented associations between drink preferences and adiposity. The other two studies had included drink items in their questionnaires but incorporated these items into composite food categories (‘sweet foods’), using the preference rating for the assessed drink items as indicators for liking for sweet tastes (e.g. chocolate milk, soft drinks) or bitter tastes (e.g. coffee, tonic water). The only study that presented results on drink preferences specifically in relation to BMI was in a large sample (n=1233) of 10-14-year-old German youngsters (Diehl 1999). In this study, preference ratings for 14 drink items (e.g. apple juice, orange juice, cola, lemonade, hot chocolate, fizzy water, fruit tea, herbal tea, milk coffee, vegetable juice) were measured on a 5-point Likert scale. None of the preferences for drink types were significantly associated with self-reported BMI z-scores. However, this study is relatively old (1999), and beverage consumption patterns have changed dramatically - especially amongst this age group. Children and adolescents have greater access to a range of drinks than ever before, and it is possible that in the current environment higher preference for SSBs is more likely to lead to actual consumption, than previously. It is therefore possible that an association would be observed in more contemporary samples.

It was also striking that this was the only study that investigated food and drink preferences in relation to BMI in young adolescents. Participants in this study were 10-14 years old; no studies were found that investigated the food and drink preferences of older adolescents. Adolescence, defined by the WHO as lasting from the age of 10-19 years, is an important developmental stage during which individuals make great gains in independence and autonomy, and increasingly take ownership of their behaviour as they make the transition from child to adult (Sacks 2003). This phase offers an ideal opportunity for cost-effective behavioural interventions as health behaviours (e.g. healthy food and drink preferences) adopted at this age are likely to persist into adulthood (Nicklaus et al. 2004; Kelder et al. 1994), benefitting health outcomes in the long term. There is a clear need for research on adolescent food and drink preferences in relation to adiposity, and future investigations should direct their attention on this age group.

An unexpectedly small number of studies investigated the associations between food and drink preferences and BMI in large representative samples, and many studies had very small samples sizes (e.g. Fisher & Birch 1995: n=18; and both Ricketts et al 1997 and Duffy et al 2009: n=88). Large representative samples are necessary for establishing reliable relationships between food and drink preferences and adiposity, and for allowing generalisation of findings to the wider population. Large scale studies in this field of research will be required to limit the influence of outliers, to produce statistically significant results, and to improve generalizability of findings to target populations.

Ten of the 14 studies relied on food and drink preference questionnaires, generally recording preference ratings on Likert scales, with response options ranging from 3 to 9 points (e.g. ‘like a lot’ to ‘dislike a lot’). Questionnaire-based measures allow for larger scale data collection; there is a need for data to be collected using consistent standardised questionnaires so that findings can be compared. Additionally, 12/14 of the studies were cross-sectional which precludes the ability to draw any conclusions on the causal direction of the observed associations. Nevertheless, these studies are important for establishing relationships where they are unknown.

There are likely multiple confounders whose presence affects the relationship between food and drink preferences and BMI, such as ethnicity, socioeconomic status and genetics. Distortion of the association between food and drink preferences and adiposity cannot be totally accounted for by controlling for potential confounding variables in standard analyses of unrelated individuals, but family-based studies provide a useful means of doing this (Thomson 1995). In particular, studies of identical twins discordant for a phenotype offer a unique opportunity to study the association between two traits, completely unconfounded by genetic influence on both (because they are genetically identical), and aspects of the environment that are completely shared by twin pairs, such as parenting, socioeconomic position, and ethnicity, and so on (Zwijnenburg et al. 2010). This informative method has been used to study other phenotypic relationships, e.g. childhood exposure to anaesthesia and cognitive development (Bartels et al. 2009), but not for BMI and food or drink preferences.

1.1.5 Aetiology of food and drink preferences

It is well established that individual differences in food and drink preferences are shaped by several cultural and social influences, but the pervasive variation in preferences indicates that individual level factors are important in driving preferences as well. A graphical representation of key influence on food and drink preferences in young adults is shown in **Figure 1.1** below. Genetic variation is a fundamental driver of all studied complex behavioural traits; in fact, heritability typically accounts for between 30% and 50% of the variation in behavioural traits (Polderman et al. 2015; Plomin et al. 2013). It is therefore likely that genetic influences are important shapers of an individual's food and drink preferences, as well as their environment. Understanding the relative contribution of environmental and genetic influences on variation in food and drink preferences is important for informing interventions to modify preferences. For example, finding a larger contribution from the environment may suggest that they are strongly amenable to change. In addition, influences on food preferences can vary with age, and it is useful to understand how individual differences in preferences are influenced at different developmental stages.

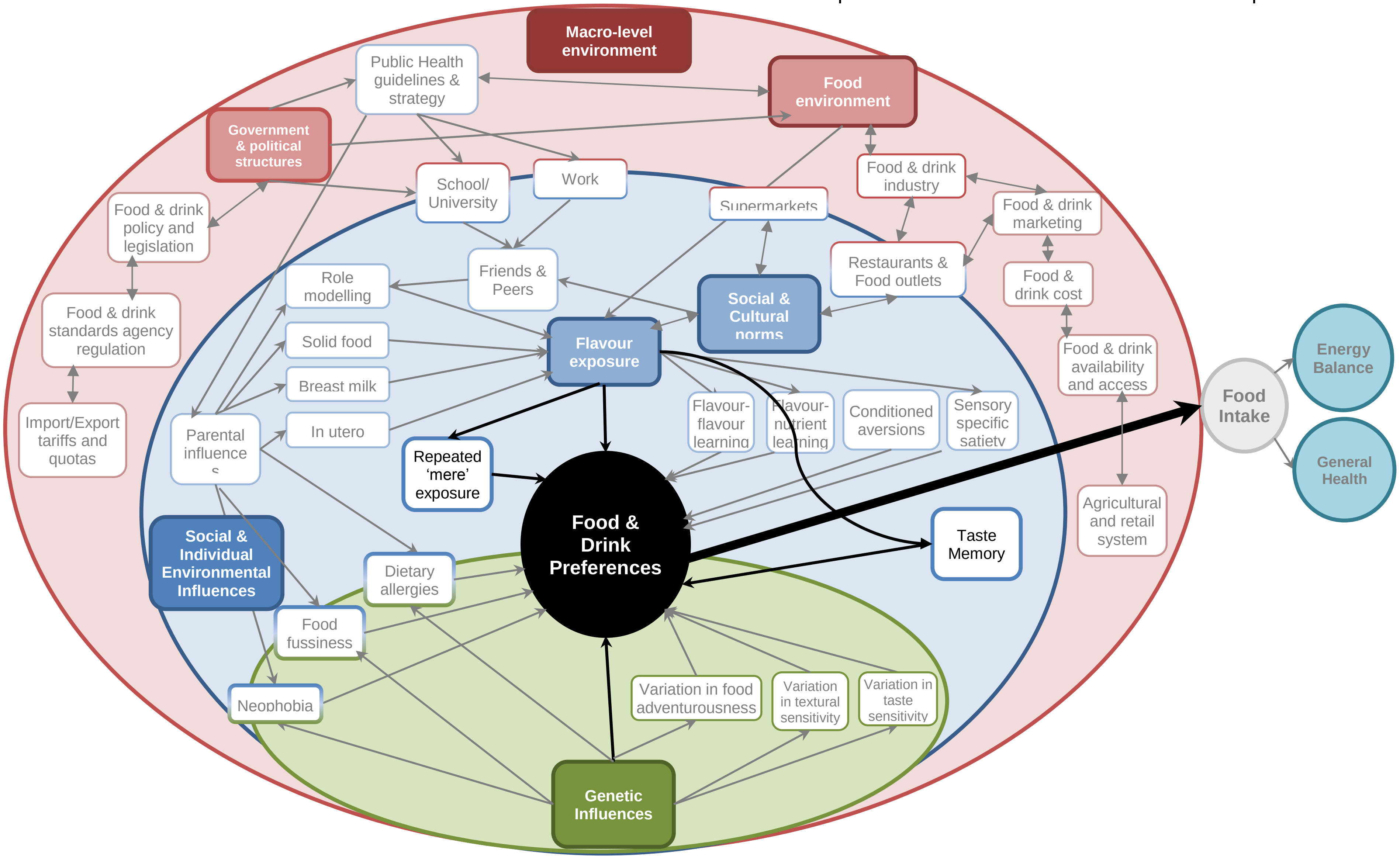


Figure 1.1 Important in influences on food and drink preferences in young adults
Adapted from (Fildes 2014)

1.1.5.1 Behavioural genetic designs studying food and drink preferences

Behavioural genetics is the field of research concerned with establishing the relative importance of genetic and environmental contributions to *individual differen*ces in behavioural characteristics (Plomin et al. 2013). The main tool of behavioural genetics is families; if family members who are more closely related genetically are also more similar on the behavioural trait of interest, *variation* in the trait is assumed to have some genetic basis.

Comparisons are typically made between parents and children, or siblings to estimate the 'familiality' of behaviour (the extent to which family members resemble one another for a given characteristic). Correlations between family members for a particular trait reflect both contributions from shared genes, and/or contributions from aspects of the environment that families share in common (e.g. socioeconomic status and aspects of the home family environment). Essentially, familiality provides an indication of the extent to which a behaviour or trait 'runs in the family' but it is not possible to separate out the genetic from the shared environmental influences by comparing standard family members – the two are always confounded. This problem cannot usually be circumvented even by comparing family members who differ in their genetic relatedness (e.g. siblings and half siblings, or siblings and cousins); because family members who are less closely related genetically tend also to share less of their environments. Twins offer an ideal opportunity to do this.

The basis of the twin method is to compare the degree of resemblance between identical or monozygotic (MZ) pairs who share 100% of their genes, with that between non-identical or dizygotic (DZ) pairs who share approximately 50% of their segregating genes. The more similar MZ pairs are for variation in a trait relative to DZ pairs, the stronger the genetic contribution to the trait. This inference is based on the assumption that MZ twins are twice as similar genetically than DZs (Plomin et al. 2013). The statistic derived to estimate the genetic contribution is called 'heritability', and can be thought of as an index of the genetic effect size; heritability quantifies the proportion of trait variation attributable to genetic variation. The remaining variance is that which is influenced by the environment, and twins allow the environmental variation to be further partitioned out into the shared environmental influences (e.g. any factors that make two twins in a pair more similar to one other beyond the effects of genetics, such as the home, shared family practises and schooling experiences) and non-shared environmental factors (e.g. experiences or influences that account for any differences between twin pairs such as having different friends or bouts of illness experienced by one twin only). The non-shared environmental component of variance also includes measurement error).

Estimates derived from twin studies are population- and time-specific; meaning that the influences observed in a sample cannot be assumed to reflect influences in other populations or at different ages. Importantly as well, the estimates do not infer risk; i.e. heritability does not indicate the likelihood that something is going to happen. Rather, estimates from twin studies indicate the contribution of genetic and environmental variation to individual differences in each trait. There have been several family and twin studies of food and drink preferences. These are reviewed separately below.

1.1.5.2 Family studies investigating food and drink preferences

Several studies have investigated whether food preferences run within families; but most of these studies are relatively outdated. In reviewing the literature, research into family correlations for taste preferences have received remarkably little attention. Only one study on sweetness taste preferences, measured using an objective Sweet Preference Inventory test, between Brazilian mother/child pairs (n=255 pairs; 4-5 years of age) found a weak but significant correlation ($r=0.12$; $p<0.05$) (Maciel et al. 2001).

On the other hand, a meta-analysis on family resemblance for food preferences by Borah-Giddens and Falciglia (1993) reported significant but small positive correlations for food preferences between mother-child pairs and father-child pairs (both, $r=0.19$) (Borah-Giddens & Falciglia 1993). These findings were based on the pooled results from five of the seven identified studies that were eligible for quantitative analysis. Since the analysis, one further study on the familial resemblance of food and drink preferences has been published (Skinner et al. 2002), resulting in a total of eight studies that will be discussed in detail in the following section. The studies are also summarised in **Appendix A2**.

Children and adolescents sampled in the studies varied substantially in age, with five studies focusing on toddlers and school-aged children (2-11 years) and three studies on high school and college-aged adolescents (17-24 years). In 7/8 studies, participants (or parents of young children) were instructed to rate their liking of food and drinks on either VAS or Likert scales, with response options ranging from 3 – 9 points. Only one study of toddlers required participants to rank the order of their liking for eight different food items (Birch 1980). Food and drink preference measures were mainly collected by self-report, apart from one study where the mother of the family completed the food preference questionnaire on behalf of her partner and offspring (Pliner & Pelchat 1986).

Three studies used structured interviews to ascertain the food preferences (Birch 1980; Weidner et al. 1985; Skinner et al. 2002), and one study used interviews for the children only (parents were asked to complete the questionnaire by themselves) (Burt & Hertzler 1978). Four other studies relied on questionnaires that were handed out to the participants (Pliner & Pelchat 1986; Logue et al. 1988; Pliner 1983; Rozin et al. 1984). Food preferences were measured using heterogeneous food classifications, with four studies assessing preference ratings of specific food items (Pliner & Pelchat 1986; Birch 1980; Weidner et al. 1985; Rozin et al. 1984), and four studies assessing a wider range of items from broad food groups (i.e. a couple of items representative of fruits, vegetables, protein etc.) (Logue et al. 1988; Pliner 1983; Burt & Hertzler 1978; Skinner et al. 2002). For ease of interpretation the findings for individual food items and food groups are reviewed separately.

1.1.5.2.1 Family studies investigating individual food item preferences

A study by Pliner and Pelchat (1986) showed that preferences for 139 individual food items were positively correlated among parents and children (24-83 months) in a sample of 55 Canadian families. In this study, mothers were instructed to complete food preference questionnaires for all family members, rating food preferences on a 6-point Likert scale. Effect sizes were relatively small ($r=0.2$ for both child-mother pairs and child-father pairs), but correlations were stronger ($r=0.5$) between siblings (Pliner & Pelchat 1986). One notable limitation with this study is that the mother was the respondent for everyone, which may have introduced bias. It is likely that a busy mother might think that all family members equally like a food that she serves, resulting in imprecisions of the mother-rated scores.

In an interview study on young children (n=128; 35 months - 5 years and 11 months), both parents and their offspring ranked their own liking of eight food items (e.g. vegetables, fruits, sandwiches) (Birch 1980). Only 10% of the mother-child preference correlations and 6% of the father-child preference correlations were significant ($p<0.05$) due to the limited power of the study design. As part of a family-based intervention to improve dietary behaviours within the home environment, food preferences for five different low-fat items (rice, yogurt, refried beans, broccoli, and fish) were recorded on a 5-point Likert scale via phone interview in a sample of 5-8 year olds and their parents (n=30 families). Resemblance of mothers' and children's food preferences for each of the assessed foods were not significant ($r= -0.09$ to 0.28, $p>0.05$), although the sample was likely too small to detect small correlations (Weidner et al. 1985). The observed correlation was substantial but not statistically significant due to the limited sample size, clearly underlining the need for larger samples to undertake this research.

A study that looked at familial resemblance in food preferences in an older sample of college students (n=34; age ranging from 17-23 years) and their parents, did not find many significant similarities between parents and their children (Rozin et al. 1984). There was a significant correlation between mothers' and children's liking for black olives (r=0.50, p<0.05), but preference scores for 21 other food items (e.g. radishes, beef, liver, yogurt, diet soda) were not significantly correlated. However, again, the sample was very small and therefore underpowered to detect small correlations.

1.1.5.2.2 Family studies investigating food group preferences

Food preference correlations for 32 food items grouped into four food groups, between parents and their primary school-aged children (5-6 years) were measured in the children by a researcher-lead interview using a 4-point Likert scale (Burt & Hertzler 1978). Parents were asked to complete a food preference questionnaire that featured the identical food items included in the children's interview. However, the authors did not report any results on the statistical significance of the overall parent-child food preference resemblance scores.

As far as I could determine, only one family study has been conducted since the 1993 meta-analysis. This showed that mothers' and their 8 year olds' (n=70 mother-child pairs) food preferences were moderately and significantly correlated (Skinner et al. 2002). Correlations were strongest for liked foods (r=0.37, p<.01), disliked foods (r=0.25, p=0.03) and for foods that had never been tasted (i.e. mothers and children had often both not tried a food) (r=0.24, p=0.04). Ninety food and drink items were categorized as 'liked', 'disliked' or 'not previously tasted' based on ratings collected during a researcher-lead interview in which the children had been asked to rate their liking (on a 3-point Likert scale) or unfamiliarity for the items. Food preferences also tracked longitudinally but maternal-child correlations were only compared at one time point. The authors concluded that familiality of food dislikes likely arises from mothers failing to introduce their children to foods that they themselves do not like.

Logue et al found small to moderate correlations (r=0.24-0.55; p<0.001) across food preference scores measured on a questionnaire for seven food categories ('milk products', 'green and yellow vegetables', 'citrus fruits', 'potatoes, vegetables, and other fruits', 'meat, poultry, fish, and eggs', 'bread, flour, and cereal', and 'butter and margarine') on a 9-point Likert scale in family-units of high school students (n=77; mean age: 15.7 years) and their parents (Logue et al. 1988). Further results from a study on college students' (n=105; 19-24 years) and their parents' food preferences found significant but small positive correlations (r=0.25 for mother-child pairings; r=0.25 for father-child pairings) for 47 food item preference scores that had been reduced to four broad but unspecified food groups (the author did not report what was included in each group) (Pliner 1983). Interestingly the researchers established that such findings were partially replicated when food preference scores of the students were correlated with random adult food preference scores (so called 'pseudo parents'). Pliner suggests that observed familiality for food preferences may be the result of a shared wider environment, e.g. foods on offer in local shops.

Generally, the results from family correlation studies show small to moderate correlations between parents and children for food preferences (ranging from r=0.2 to 0.5). Most studies have been very small, and null findings were often reported for small correlations; which is probably due to insufficient power to detect significant effects. One study by Pliner & Pelchat (1986) showed considerably larger correlations between siblings than for parents and children. This may reflect the fact that siblings are closer in age. Genetic influences vary with age and so effect sizes may be more similar among more developmentally matched individuals. There may also be more environmental influences that are shared by siblings than by parents and children (e.g. the school setting). Food preferences vary with age as well, and this has considerable implications for resemblances between parents and their children. Future longitudinal studies would be able to show how concordance in food preferences between parents and offspring change as children mature into adults.

Importantly, the period of time during which most of these results were published suggests that all of the family studies on food preferences may be out-dated – only one study has been published since the 1993 meta-analysis. Food preferences change considerably at a population level over time, as different trends come in; estimates of familiality might therefore vary with time as well, so more present-day studies are needed.

1.1.5.3 Twin studies investigating food and drink preferences

Increasingly evidence for the heritability of food and drink preferences is coming from studies using twins. Like family studies, twin studies have focused on investigating preferences for individual food items, as well as for broader food groups or dietary patterns. Earlier (and smaller) studies have tended only to report intraclass correlations for MZ and DZ pairs; a higher MZ than DZ correlation provides an indication that there is a genetic contribution to preferences for that food or drink type. However, more recent and larger studies have used more sophisticated modelling techniques that have enabled calculation of estimates for each of genetic, shared environmental and unique environmental influences. Studies reporting these estimates are easier to compare. Twin studies of preferences for individual items and food groups are reviewed separately below and summarized in **Appendix A3.**

1.1.5.3.1 Twin studies investigating individual food item preferences

Earlier twin studies of food and drink preferences tended to focus on individual food items. Findings indicated that the strongest genetic influence was frequently observed for preferences for food or beverage items with known pharmacological properties, e.g. alcohol and caffeine; or spicy foods. However, genetic effects were not demonstrated in all studies, and the magnitude of effect sizes varied (Reed et al. 1997).

For instance, in a sample of 35 9-18-year-old twin pairs, preference ratings for 17 individual foods were tasted and rated on a questionnaire (Falciglia & Norton 1994). The MZ correlations were significantly higher than those for the DZs (indicating a genetic contribution to variation in preferences) for six out of the 17 items (orange juice, broccoli, cottage cheese, chicken, cereal (sweetened), and hamburgers). Findings were also mixed for another adult twin study, which suggested that there was a genetic contribution to variation in liking for eight out of 24 individual food and drink items (broccoli, bacon, strawberries, green beans, unsweetened apple sauce, unsweetened orange juice and unsweetened grapefruit juice) (Krondl et al. 1983).

A genetic contribution to variation in liking of spicy food has been suggested in an early British adult twin study by Faust (Faust 1974). Food preferences were measured by asking about any spontaneous food likes or dislikes (using the open-ended question “Is there any food which you really dislike?”), and ‘Do you like…’ for seven food items (melon, tomatoes, parsnips, cheese, yoghurt, anchovies, liver) which twins responded to with either ‘yes’ or ‘no’. Spicy/foreign foods were the only spontaneously mentioned food dislikes for which correlations were significantly higher for MZs (n=48 pairs) than DZs (n=48 pairs). A heritable basis to the liking of melon was also shown in another study of 37 young adult twin pairs (Rozin & Millman 1987). In this study, MZ correlations were also significantly higher than DZ correlations for liking of chilli pepper, suggesting a genetic component to variation in preference for this spicy food. Food preferences were measured in a telephone interview and participating twins were required to rate their liking for the food items on a 9-point hedonic scale. The choice of food items included on the questionnaire was based on previously observed high variability in the liking for these items (Rozin et al. 1984).There were significant differences in resemblance between MZ and DZ pairs for the liking of plain yoghurt and peppermint, but the DZ correlations were negative making it difficult to interpret the findings. Results were mixed for the other 11 food items (radishes, beef liver, black olives, black coffee, soft-boiled eggs, lima beans, liverwurst, raw onions, sugar, and lemon).

In a more recent and larger study, twins were recruited from two Scandinavian twin registries; one in Denmark (n=585 pairs) and one in Finland (n=2109 pairs). Hasselbalch and colleagues (2010) focused on preference for rye bread compared to white bread. Twins in the cohorts were asked to complete a food frequency questionnaire, specifically investigating the preference of choosing certain bread types in their typical diet (with response options ranging from ‘Never’, to ‘3-4 times/week’, to ‘≥8 times/day’). Sex-specific analysis was undertaken and heritability estimates for preference for rye bread was 27% and 37% for females and males respectively in the Danish sample, and 29% and 48% for Finnish females and males. Overall, no shared environmental influences were observed, with all remaining variation in preference for rye bread being explained by aspects of the environment that were unshared by twin pairs (Hasselbalch et al. 2010).

It is likely that the observed inconsistencies in these studies partially result from the small sample sizes and varying demographics of the twins across the different studies; but also, from the different types of food and drinks for which preferences were measured, and differences in measurement methods. Twin studies require large sample sizes and sizeable variation for a trait for reliable parameter estimates to be established (Martin et al. 1978).

1.1.5.3.2 Twin studies investigating food group preferences

Twin studies that have aggregated foods into categories or groups have demonstrated more consistent evidence of moderate genetic influence on variation in food preferences. One of the earliest studies collected self-reported preference data on 24 individual food items grouped into ‘staple foods’, ‘fruit’ and ‘vegetables’ on a 3-point Likert scale, in a sample of American teenage twins (n=219 pairs; 13-16 years old) (Smith & Vandenberg 1965). Mean within-pair resemblance for MZ pairs was double the within-pair resemblance index of DZ pairs for the liking of both fruit and vegetables, suggesting genetic contributions to variation in preference for these food groups. In a similar approach, Törnwall et al (Törnwall et al. 2014) categorized 85 food items into two groups; `basic foods' and `adventurous foods' (encompassing more sour and pungent items). Genetic factors strongly contributed to being a liker of either 'basic' or 'adventurous' foods groups (72%) in this sample of 22-year olds (n=140 pairs), with remaining differences attributable to unique environmental influences.

A study of British adult twins (n=324 pairs; mean age of 55.6 years) focused on liking of 'sweet foods', measured using 6 items; dessert, sweets, pastry, ice-cream, hard candy and chocolate (Keskitalo, Tuorila, et al. 2007). Variation for liking of 'sweet foods' was roughly equally explained by genetic (54%) and non-shared environmental effects (46%).

The strongest evidence for genetic influence on food preference has been provided by two recent twin studies conducted in paediatric British twin cohorts. Both studies administered extensive food preference questionnaires and used Principal Components Analysis to examine underlying food preferences groups. In the first of these studies, heritability estimates for preferences for four food groups (fruit, vegetables, protein and desserts) were measured in a subgroup (n=214 pairs) of twin children from the Twins Early Development Study (TEDS), a large population-based birth cohort of British twins (Breen et al. 2006). Parents reported on their 4-5-year-old twins' preferences for 72 food items. Heritability was high for liking of protein foods (78%) and moderate for fruit (51%) and vegetables (37%). In comparison, lower heritability for liking of dessert foods (20%) was observed. Shared environmental effects on food preference variation were highest for desserts (62%), modest for vegetables (46%) and fruit (36%), and lowest for the liking of meat and fish (14%). The authors hypothesised that these results suggested that genes exert their strongest influence on preferences for the most nutrient-dense food groups (e.g. vegetables and protein foods) as these groups may be the most homogenous in terms of taste and texture. The significant influence of the shared-environment may reflect the fact that there was a common rater (i.e. the mother rating food preferences on behalf of her child). However, it is more likely these findings are a reflection of the young age of the sample, with most eating occasions under parental control at this early age, and these influences are represented by the shared-environmental estimates.

The second paediatric study considering preferences for a range of food groups was the largest of its kind (MZ pairs: n=458, DZ pairs: n=872), and used data from the Gemini study. Parents reported on their 3 year old twins' preferences for 114 food items, of which 75 food item preference scores were retained in the final analysis (Fildes, van Jaarsveld, Llewellyn, et al. 2014). Principal Components Analysis identified six empirically derived food groups that reflected traditional food categories (vegetables, fruits, protein, dairy, starches, and snack foods). Heritability was significantly higher for liking of vegetables (54%), fruits (53%) and protein (48%) than for liking of snacks (29%), starches (32%) and dairy (27%)., The moderate to high heritability estimates for fruit and vegetable liking were comparable to the heritability estimates seen in the previous Breen et al. study in twins of a similar age (Breen et al. 2006). Heritability for the preference for protein foods however was substantially lower in the Gemini Study sample compared to the slightly younger TEDS sample (78%). Importantly, unique environmental influences were low, with estimates ranging from 11% for vegetables, snacks and starches, to 19% for dairy foods. Most of the remaining variance was explained by the shared environment. This was an observation in both the TEDS and the Gemini data. This is again unsurprising as at this young age most eating occasions occur within the home, which is shared between twin pairs. Also what foods the children are given is probably decided by the mum/parent, not by the children. Lastly, it is clear that twin studies have either focused on adults or young children and few studies (Smith & Vandenberg 1965; Falciglia & Norton 1994) have looked at genetic and environmental influences on food and drink preferences in adolescents.

1.1.5.3.3 Twin studies on drink preferences

In comparison to twin studies on food preferences, research into the genetic and environmental influences on drink preferences is scarce. Twin studies that have looked into drink preferences have mostly focused on alcohol and coffee. Studies of both caffeine and alcohol dependency have shown that both traits are heritable, with heritability estimates ranging from 36% - 58% for caffeine (Yang et al. 2010) and 30% - 70% for alcohol dependency (Agrawal & Lynskey 2008). To date only two twin studies have explored genetic and environmental contributions to individual differences in preferences for a beverage, and both studies have focused on preference for coffee (Luciano et al. 2005; Vink et al. 2009). Both studies defined coffee preference as a relative preference; indexed as the proportion of cups of coffee consumed per day relative to the number of cups of tea. Both studies suggested that a moderate proportion of variation in coffee preference was attributable to genetic differences (42% in Luciano et al and 62% in Vink et al).

However, the genetic components affecting variation in coffee preference are likely to be very different to those underlying food preferences, and preferences for other non-caffeinated drinks. Coffee is a regular source of caffeine for around 80-90% of the world's population and it is widely enjoyed for its psychoactive effects (Drewnowski 2001). However, genetic differences in the ability to metabolise caffeine may influence heritability estimates for coffee preference, as individuals highly sensitive to caffeine may report lower liking for coffee due to the unpleasant psychoactive effects rather than the taste. Genetic and environmental contributions to preferences for other beverages need to be established.

1.1.5.4 Summary of the findings of the current literature on genetic and environmental influences on food and drink preferences

Behavioural genetic studies have found robust evidence for genetic influences on food and drink preferences in adults and children but the aetiology of these characteristics is understudied in adolescence. A key observation was that the aetiology of most drink preferences has not yet been explored, with only two studies investigating the genetic and environmental influences on variation in the liking for coffee. Given the increasing concern over consumption of SSBs, this is an important gap in the literature that needs to be filled.

Very few studies (other than the two paediatric studies) have explored preferences for a large range of food items or groups, and very few have taken a 'data driven' approach to deriving food groups (e.g. using methods such as Principal Components Analysis). Additionally, many of the sample sizes were small but large samples are needed to produce reliable estimates of the relative importance of environmental and genetic factors in shaping food and drink preferences in the general population.

1.1.5.5 Molecular genetic research to identify common genetic variants

Finding moderate heritability estimates for food and drink (i.e. coffee) preferences from twin studies has led to an interest in identifying the specific genes underlying variation in these preferences. Molecular genetic studies offer a relatively new technique for examining genetic variation in relation to food and drink preferences. In particular, genome wide association studies (GWAS) seek to identify common genetic variants (in the form of single nucleotide polymorphisms, SNPs) associated with variation in complex traits (Zeng et al. 2015). A GWAS by Eriksson et al (2012) found a significant association between the rs72921001 SNPs and individuals who reported a 'soapy' taste when eating coriander (n=26,691; mean age range: 43.8 – 49 years) (Eriksson et al. 2012). Further evidence linking SNPs with specific food and drink preferences was reported by Pirastu et al (2012). In this central Asian population-based study (n=478; age range: 8-84 y), significant associations between variants in the TAS1R2 gene and liking of lamb, white wine and vodka were found. Furthermore, variants in the PCLB2 gene were associated with liking of tea, whilst liking for beetroot was linked to variants in the ITPR3 gene (Pirastu et al. 2012).

The most recent large scale GWAS (n=4611; mean age range: 39.1-53.2 y) of food preferences offering the most robust analysis so far, was published in April 2016. It used a two-step meta-analysis including three distinct populations from Italy for the discovery stage, and two samples from the Netherlands and Central Asia for the replication, to identify common genetic variants that explain variation in liking for 20 specific foods, belonging to 4 categories (vegetables, fatty, dairy, bitter). Food preferences were measured using an operator-administered food preference questionnaire scoring food likes and dislikes using on a 9-point Likert scale. Overall,15 SNPs were identified that explained variation in liking for 12 foods (Pirastu, Kooyman, Traglia, et al. 2016). It was noteworthy that none of the genes coded for taste or olfactory receptors, indicating that genetic influence on food liking may be more complex than simple taste or smell perception (Reed & Knaapila 2010) . Genetic influence may be mediated by mechanisms such as perceived hedonic signalling upon ingestion of certain foods, or more psychological factors such as food neophobia (Cooke et al. 2007).

The relatively high cost of genotyping chip technology and processing of SNP microarrays associated with these studies has meant that findings arising from this field have been undertaken in limited sample sizes, and need to be replicated in larger and more diverse population groups (Hong & Park 2012; Zondervan & Cardon 2007). However, the effect sizes of SNPs tend to be very small, and studies are rarely large enough to be able to find significant associations. Twin studies therefore remain an important source of information about the relative contribution of genetic variation to individual differences in food and drink preferences.

1.1.5.6 Research to identify environmental influences on variation in food and drink preferences

Twin studies have also indicated that a sizeable proportion of variation in food preferences is shaped by environmental influences. As one might expect, aspects of the environment completely shared by twin pairs plays a more important role in shaping young children’s preferences, but aspects of the environment that are unique to each individual twin (unshared between them) seem to play the most important role in adult food preferences. For the first few years of life, parents are the primary providers of food to their young child. Once children start school they encounter increased opportunity for eating without the parent. The context also becomes more social; children of comparable age tend to eat together at school, opening the opportunity for peer influence. The relative importance of the home and of parental influence may therefore begin to decrease as children become more independent from the family. In adolescence, the individual develops his or her own identity, as well as autonomy, and eating occasions take place increasingly outside of the home environment (Nu et al. 1996; Pelletier et al. 2014; Higgs & Thomas 2016). Social or peer pressure can act to increase the liking or disliking of certain foods and has noticeable implications for adolescent eating behaviours; especially if observation of friends' preferences influence their own (Houldcroft et al. 2014; Robinson 2015). There are only two previous twin studies of food and drink preferences of adolescents (Falciglia & Norton 1994; Smith & Vandenberg 1965), and it is still unclear when the apparent transition from the shared to the unique environmental influence occurs.

There is a wealth of research into the specific environmental factors that influence food preferences at different ages. It is beyond the scope of this review to include them all here, but one of the most important influences is 'exposure'. Exposure to flavours influences taste preferences or flavour aversions, depending on the context and consequences of the experienced stimuli. Exposure results in increased familiarity with a flavour or food. Most children display a preference towards the foods that they are familiar with and an aversion towards novel foods (Birch 1999).

Other environmental influences that shape food and drink preferences include the higher motivation of young children to try an unfamiliar food if they have previously seen an adult eat it, i.e. modelling of food intake (Addessi et al. 2005). There is also evidence that peer-modelling is important, and that this is especially important for adolescent populations (Salvy et al. 2012; Stok et al. 2016). Numerous studies have shown that the more familiar (Harper & Sanders 1975) and similar the model is in terms of age (Duncker 1938) or gender to the individual observing the behaviour (Frazier et al. 2012), the higher the likelihood for increased food acceptance and higher preference ratings for the target food and drink items.

These mechanisms form the basis by which taste preferences are learned throughout a person's life, as they encounter and taste a variety of food and drinks, with persistent effects later in life (Bartoshuk & Beauchamp 1994).

1.2 Summary of food and drink preference research

Obesity is a preventable major public health burden across the globe. Food and drink preferences are strong determinants of actual food and drink intake, and individual differences in preferences for energy-dense foods or drinks are therefore implicated in obesity susceptibility. Following the reviews on both the relationship between food and drink preferences and adiposity, and the aetiology of food and drink preferences, there are important gaps in the literature.

Historically the study of the genetic and environmental influences on variation in food and drink preferences has nearly exclusively focused on foods. However, beverages are increasingly becoming significant contributors to individual energy intake as the availability and diversity of soft drinks, fruit juices and other sweetened beverages continues to grow. Nevertheless, preferences for drinks have not yet been integrated into most of the recent studies on variation in food and drink preferences. Commonly used food preference questionnaires tend only to include limited items relating to drink preferences (e.g. fruit juice, coffee), if any at all. Additionally, it has been proposed that food and drink preferences may differ substantially with respect to their aetiology. For instance, Gareth and Griffins (1998) suggested that the invigorating effect and avoidance of caffeine withdrawal symptoms may reinforce liking for fizzy caffeinated drinks, rather than characterise an underlying affinity for the flavour of the drink (Garrett & Griffiths 1998). There is a need for studies to investigate the relative contribution of genetic and environmental factors to drink preference variation to clarify whether the aetiology of food and drink preferences are similar or different. This will inform the development of public health interventions for the modification of preferences. Family and twin studies provide a powerful method for disentangling the relative importance of genetic and environmental influences on variation in food and drink preferences.

In general, the relationship between food and drink preferences (as opposed to actual *intake*) and adiposity is a remarkably understudied area. No study has examined the relationship between food and drink preferences and adiposity in older adolescents. However, adolescence is an important developmental stage when individuals make the transition from childhood to adulthood; and the relationships may therefore vary during this phase. In addition, most studies exploring the relationship between food preferences and adiposity have focused on foods high in sugar and/or fat, but liking for other everyday food groups such as vegetables, meat and fish, starches or dairy products have been largely neglected. This is an important oversight because it is possible that preferences for different types of foods can either predispose to or protect against excessive adiposity. Understanding these relationships is important for public health initiatives. Importantly, the relationship between preferences for different types of drinks and adiposity is also unknown; despite the rising concern about increases in consumption of energy-dense drinks, especially among adolescents. Studies have largely been hampered by small unrepresentative samples, limiting generalisation to the wider population

Family and twin studies have shown that variation in food and drink preferences is influenced by both genetic and environmental factors. However, these traits have mainly been researched in adult and children, and examination of the aetiology of food and drink preferences in an adolescent sample would further our understanding of the aetiology of these traits across the lifespan. Given that adolescence is an important developmental stage marked by increased independence, the relative influence of genes and environment on food and drink preferences may differ considerably from those observed during either childhood or adulthood. A striking observation was the importance of the shared environmental influences on the food preferences of young children, replaced by the unique environmental effects on the preferences of adults. It would be useful to establish the relative importance of these two sources of environmental influence during this important transitional phase from childhood to adulthood. The findings have important implications for public health initiatives to change them: if preferences are shaped more by the shared environment, interventions should target families and the home; if preferences are shaped more by the unique environment, interventions might fare better if aimed at the wider environment outside of the home. Furthermore, the aetiology of drink preferences has been largely neglected in all age groups.

As was seen in the literature, there is considerable environmental influence on food and drink preferences, indicating that these traits may be malleable. A summary of the evidence on the modification of food and drink preferences is presented in **Chapter 7**.

There is tentative evidence to suggest that individual preference for various nutrients (e.g. fat, saltiness) in foods can selectively be modified. Nevertheless, one of the most remarkable findings is that until today, no intervention has tested a taste preference modification protocol to increase the liking of a specific food or drink item in young adults or adulthood.

This thesis will focus on understanding how variation in food and drink preferences relates to adiposity in late adolescence; understanding the relative importance of genetic and environmental influence on preferences for a range of foods and drinks;

and exploring the potential for environmental modification of food or drink preferences. A detailed description of the aims of the thesis is presented in **Chapter 2**.

Chapter 2.

Aims of the thesis

2.1 Key objectives of the thesis

The first chapter of this thesis reviewed literature on the associations between food and drink preferences and adiposity, and the evidence for genetic and environmental influences on variation in these dietary preferences. Substantial evidence suggests food and drink preferences are key upstream determinants of actual food and drink intake, implicating individual differences in these preferences as putative risk factors for susceptibly to overweight.

Quantitative genetic research has identified substantial heritability of food preferences in child and adult populations, but the relative influence of the shared and unique environments appears to differ as a child matures. The aetiology of food preferences in adolescence has been neglected and there is a noticeable absence of research exploring genetic and environmental contributions to drink preferences. This is surprising given growing evidence for the causal role of energy-dense and high sugar drink consumption in overweight and obesity.

Literature exploring the relationship between food and drink *preferences* and adiposity or adiposity is inconsistent and sparse, particularly in older adolescents. Moreover, observational analysis of associations between food and drink preferences with adiposity in samples of unrelated individuals cannot take substantial genetic influences on both the exposure and the outcome into account. The haphazard and non-standardised approach to studying food and drink preferences has contributed to inconsistency in findings, and is likely explained by the lack of a gold standard assessment method, making it difficult to compare findings across studies. The study of food and drink preferences in a large sample, requires a reliable, comprehensive and age appropriate measurement tool but such an instrument has yet to be developed for young adults.

Research into environmental influences on food and drink preferences emphasizes the importance of taste exposure (Yeomans 2009). Few studies have considered other food and drink exposures or investigated the plasticity of dietary preferences in an older adolescent population.

Given these important gaps in the literature on the aetiology of food and drink preferences and their relationship with adiposity, the specific objectives of this thesis are:

(1) To develop a food and drink preference questionnaire that comprises a wide range of food and drink items relevant to common dietary choices of older adolescents, and to broadly characterise patterns of food and drink preferences in older adolescent.

(2) To establish the relative contribution of genetic-, shared-, and unique environmental influences on food and drink preferences in late adolescence.

(3) To examine the relationship between a comprehensive range of food and drink preferences and adiposity in a large sample of older adolescents.

(4) To establish the feasibility, effectiveness and acceptability of a behavioural taste preference modification intervention to reduce the preference for, and intake of, sugar in hot beverages.

The studies described in **Chapters 3-7** address these objectives. **Study 1** (**Chapter 4**) details the development of a comprehensive food and drink preference questionnaire, which is used to investigate the structure of food and drink likes and dislikes of older adolescents. **Study 2** quantifies the genetic and environmental influences on variation in food and drink preferences. The first part of **Study 3** (**Study 3a; Chapter 6**) explores the cross-sectional associations between food and drink preferences and BMI in a large population-based sample of older adolescents. **Study 3b** (**Chapter 6**) compares the food and drink preferences of adiposity-discordant identical twins to establish the association between preferences and adiposity, unconfounded by genetic and shared environmental confounding. Lastly, **Study 4** (**Chapter 7**) is a pilot randomised controlled trial of two short taste preference modification programmes to reduce the intake and liking of sugar in hot tea in young adults that habitually consume tea sweetened with sugar.

2.2 My contributions to the research of this thesis

I developed the aims of this thesis, and refined these in discussion with my three PhD thesis supervisors - Dr Clare Llewellyn (primary supervisor), Dr Alison Fildes and Dr Lucy Cooke. The overall direction and scope of this PhD thesis was influenced by Professor Jane Wardle who sadly passed away in October 2015, a year after I had started my PhD. All statistical analyses were designed and undertaken by me.

The data for **Studies 1,2** and **3** (**Chapters 4 - 6**) were drawn from the UK's largest population-based cohort of twins, the Twins Early Development Study (TEDS). The TEDS office is based at the Social, Genetic and Developmental Psychiatry Centre, King's College London. Basic socio-demographic information was collected from parents of participating twins between 1995-1998 by the TEDS team. The main measures in this study (food and drink preferences and BMI) were collected from April to May 2015 from the TEDS twins directly via an online questionnaire. The online questionnaire data collection platform was developed in collaboration with Dr Nicholas Shakeshaft, at that time a PhD student working on the TEDS team. Professor Jane Wardle initiated contact with Professor Robert Plomin, the director of the TEDS team, and after presentation of my PhD research aims to him, he graciously permitted the dissemination of my food and drink preference questionnaire in a sub cohort of TEDS. From this point, I coordinated data collection with the TEDS administrative team (Rachel Ogden and Andy McMillan).

I developed the food and drink preference questionnaire in **Study 1** (**Chapter 4**), which included initial selection of questionnaire items and multiple focus groups with young adults at UCL. Various versions of the questionnaire were piloted with student volunteers, and in consultation with Professor Jane Wardle and Dr Clare Llewellyn, final food and drink items for the questionnaires were selected.

For the analyses in **Study 2** (**Chapter 5**), I attended a 5-day course on 'Twin model fitting' at the MRC Social Genetic and Developmental Psychiatry Centre at Kings College London. Here I received training in the basic principles of the classical twin model and the statistical software OpenMx to analyse twin data. I have additionally received advice on twin analysis from a fellow PhD student (Moritz Herle), and my supervisors Dr Clare Llewellyn and Dr Alison Fildes. I cleaned the raw TEDS data and carried out the twin analysis. I carried out further analysis of BMI-discordant MZ twin data in Study 3b after seeking twin modelling advice from Dr Frühling Rijsdijk, an expert in twin methodology at Kings College London.

I designed the RESIST intervention in **Study 4** (**Chapter 7**) in collaboration with my supervisors (CH, AF, LC). Some alterations to the intervention protocol were implemented after receiving feedback on the study design from my external Upgrade Examiner Professor Martin Yeomans. I applied for UCL ethical approval of the RESIST intervention and received approval in November 2016. I coordinated every element of the trial; starting with design of all the study materials including the smartphone based daily data collection tool, participant recruitment, and all aspects of data collection which included mail-outs, reminder e-mails and phone calls and in-person experimental psychophysical sweetness preference assessments. I cleaned and analysed all the quantitative data for RESIST. I also conducted the analyses of the smartphone application serial measurement data with additional guidance from Dr Annie Herbert, a Research Associate in Medical Statistics at the Department of Behavioural Science and Health (UCL). MSc student Sonam Verma joined in January 2017 to help with the qualitative evaluation of RESIST. In collaboration with SV, I developed the semi-structured interview protocol and SV conducted the interviews under my supervision. Interviews were transcribed by an external company (Devon Transcriptions Ltd.) and SV and I collaborated on the thematic analysis of the interviews.

I have also been fortunate to receive training in a broader repertoire of research-relevant skills by becoming involved with the Gemini Study (a large cohort of 2402 families with twins), based at the Department of Behavioural Science and Health. During my PhD, I managed many of the administrative tasks associated with running the Gemini Study, ranging from coordinating regular mail outs, database management, and implementing data safety protocols and corresponding with parents of participating twins. Every day for the past 2 and a half years I have sent daily hand-addressed birthday cards to the twins in this sample to keep them engaged in the study. All the skills and advice that I have received from Gemini PhD students and my supervisors have informed my approach to data management of both the TEDS and RESIST databases.

All the work presented in this thesis was undertaken by me, unless indicated by a footnote or otherwise.

Chapter 3.

Sampling and methodology

This thesis used data from two different samples. The data for the first three studies of the thesis (**Study 1, 2** and **3**) are drawn from a large population-based birth cohort of twins in the UK – The Twins Early Development study (TEDS).

In addition to the TEDS sample, data from an independent sample of young adults was used for **Study 4**; the Reduction of Sugar in Tea Study (RESIST). This sample was recruited from London-based universities in the beginning of 2017. Detailed information on sampling methodology, measures, and statistical analysis of **Study 4** is provided in **Chapter 7**.

3.1 Study population, measures and methodology for studies 1, 2, and 3: The Twins Early Development Study (TEDS)

3.2 The Twins Early Development Study

The Twins Early Development Study (TEDS) is the UK's largest population-based twin cohort. It is based at the Medical Research Council (MRC) Social, Genetic & Developmental Psychiatry Centre at Kings College London (Trouton et al. 2012). Established in 1994, TEDS includes a nation-wide sample of all twins born in 1994 to 1996. The original aim of the study was to investigate the genetic and environmental influences on common developmental problems. A brief overview of the flow of TEDS families since its inception is shown in **Figure 3.1**.

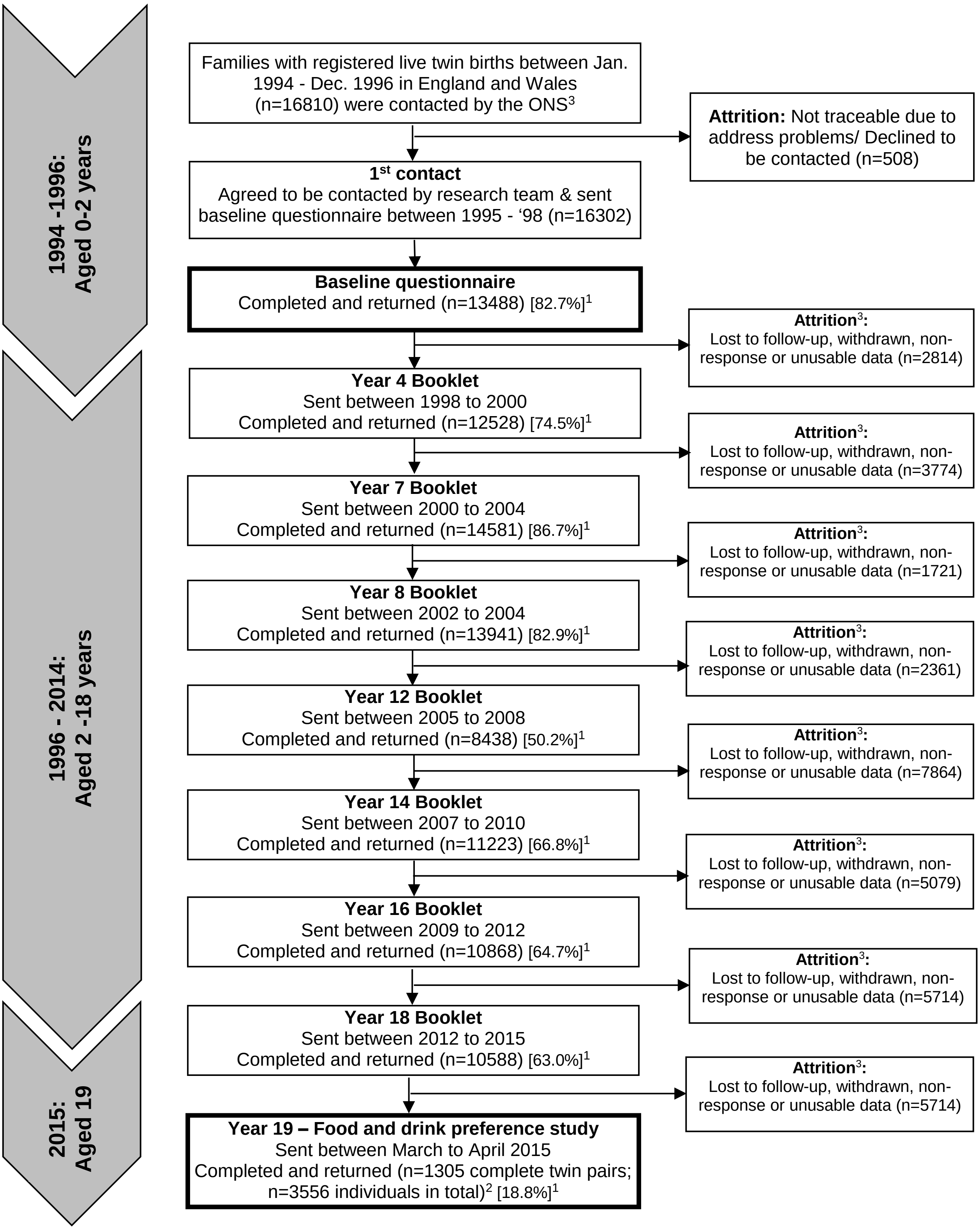
3.2.1 Recruitment process and study structure

All families with twin live births (n=16810) between January 1994 and December 1996 were contacted by the UK Office for National Statistics (ONS). The ONS sent each traceable family a recruitment pack containing an official invitation letter, a study information sheet, a reply card and a postage-paid return envelope. Of these, 16302 families agreed to participate and were sent the TEDS enrolment papers. When the twins were on average 18 months old, the TEDS team sent consenting families the baseline questionnaire which collected demographic data, information about zygosity, medical histories and asked for consent to request official hospital records concerning the pregnancy and delivery of the twins. TEDS is supported by a program grant from the United Kingdom Medical Research Council (MRC), with additional support from US National Institutes of Health Grants.

In recognition of the challenges that families with young twins experience, extensive strategies were implemented to minimize drop-out, including yearly birthday cards, small gifts, an annual newsletter, prize draws, and access to an advisory freephone line. Recently, TEDS established a twin advice panel (*The TEDS Ideas Panel*) to guide researchers on various aspects of the data collection procedures (Haworth et al. 2013). During each wave of data collection, parents or the twins themselves are given the opportunity to opt out of a certain study, or to withdraw from TEDS entirely.

For administrative purposes, the TEDS study has been divided into four sub-cohorts that reflect the typical UK school year groupings; twins born January 1994 to August 1994 comprise sub-cohort 1, twins born September 1994 to August 1995 comprise sub-cohort 2, twins born September 1995 to August 1996 comprise sub-cohort 3, and twins born September 1996 to December 1996 comprise sub-cohort 4. These sub-cohorts allow data collection to be targeted at a limited number of study participants at a time. Investigations not relying on the entire study sample can selectively send out study invitations to one sub-cohort, reducing the study participation burden on TEDS parents and twins.

Figure 3.1 Flow of families through the TEDS study



[1] Percentage of ONS sample contacted are given in square brackets [%]
[2] Only twin pairs part of the TEDS sub-cohort 3 (born from Sept. '95 to August '96) were contacted for this wave of data collection. Invitations were sent to n=6332 individuals with an overall response rate of 56.2%.
[3] Attrition calculated relative to the sample of participants that agreed to participate in TEDS at 1st contact (n=16302)
[4] Abbreviations: ONS=Office of National Statistics

3.2.2 Representativeness of the TEDS sample

The TEDS sample is largely representative of the UK population. At baseline, half of the sample was female and approximately a third of the twins were monozygotic, reflecting the distribution in the UK population. Demographic data were collected from just under 14000 households; 91.7 % of the sample were white and 35.5% of mothers were educated to A-level or higher, in keeping with UK population data (93% white; 32% of mothers attaining an A-level or higher) (Walker et al. 2004). Changes in sample demographics over time are shown in **Table 3.1**. Only minor changes have been observed, and the sample remains reasonably representative.

Table 3.1 Representativeness of the TEDS sample at various developmental stages

	Families (n)	% Response rate	% white	% A-level or higher	% Mother employed	% Father employed	% Female twins	% MZ[1]
1st contact	13 694	84.0	91.7	35.5	43.1	91.7	50.1	33.1
Early childhood (2 – 4 y)	10 150	69.3	92.9	38.1	43.4	92.3	50.9	33.5
Middle childhood (7-10 y)	8 819	59.1	93.2	39.8	46.0	92.9	51.0	35.0
Adolescence (12 – 16 y)	8 697	74.1	92.8	40.1	46.4	92.8	51.7	34.7

[1] Abbreviations: MZ = Monozygotic

A map of the geographical distribution of the TEDS sample in the year 2012, when the TEDS twins were approximately 18 years old, is shown in **Figure 3.2.** The geographical spread is roughly representative of the overall population spread of the wider population in the United Kingdom (compared to ONS data). Over the years, TEDS families have emigrated to several countries, primarily Australia (n=44), New Zealand (n=33) and the USA (n=33) which are not shown on the map.

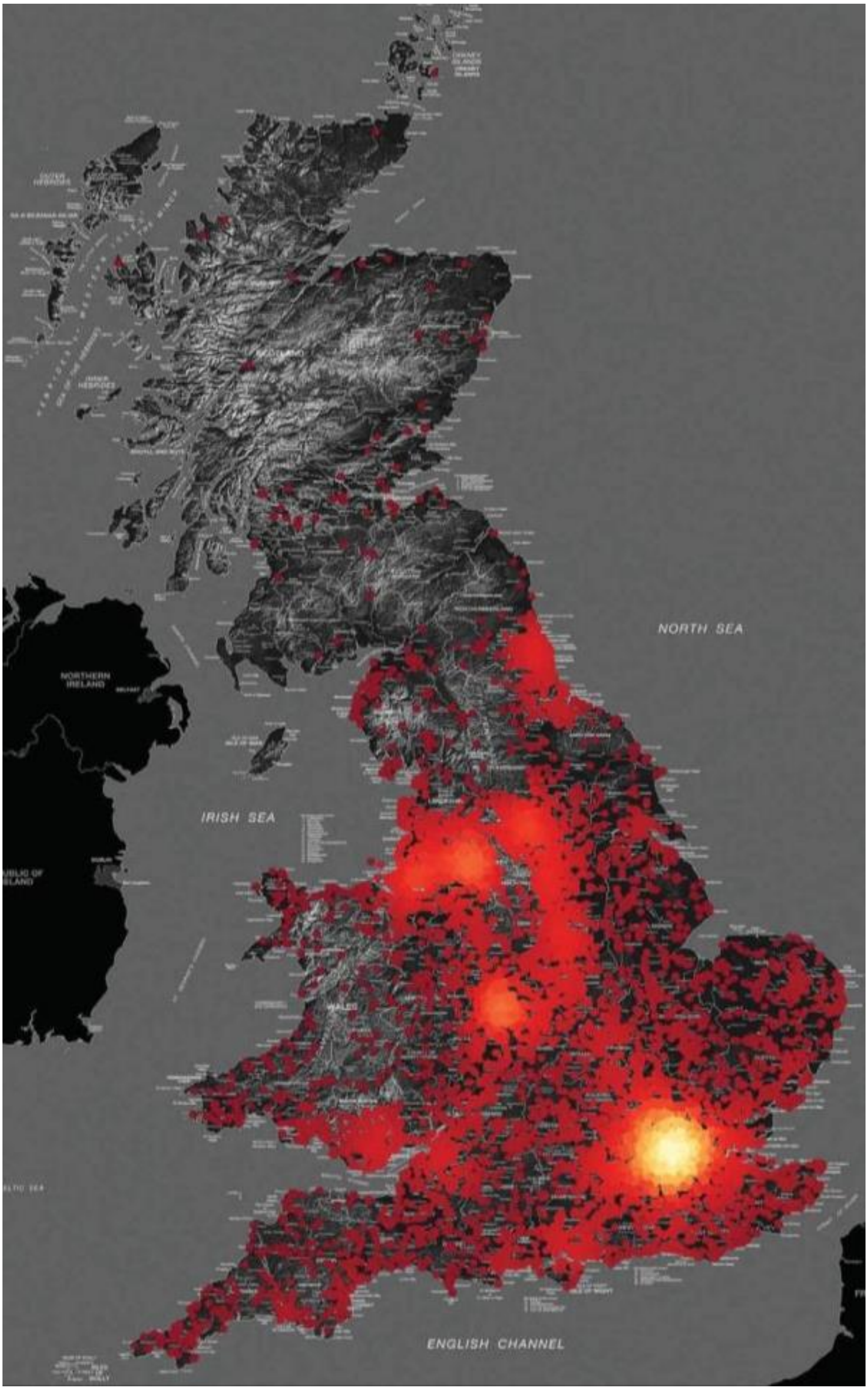


Figure 3.2 Geographical distribution of the TEDS sample in the United Kingdom (n~10 000 families)

Density of TEDS families plotted using a colour scale ranging from 'low' (red) to 'high' (yellow)
Map reproduced from (Haworth et al. 2013)

3.2.3 Data Collection

Detailed information on the TEDS data collection phases is shown in **Figure 3.1**, and described in depth in the publication by Haworth et al (Haworth et al. 2013). Data for Study 1, 2, and 3 were collected from sub-cohort 3, using an online food and drink preference questionnaire completed by the twins at the start of April 2015. Data used in Study 1, 2, and 3 were obtained in-part from the measures collected in the baseline questionnaire and the more recent food and drink preference questionnaire. Detailed information on the data collection procedure of each data wave is summarized in the following section.

1.1.1.1 Baseline questionnaire data collection in the full TEDS cohort

Baseline questionnaires were disseminated and coded from 1995 to 1998. Data collection for the baseline questionnaire was not precisely timed to coincide with an age of the twins, but twins had a median age of around 1.6 years when the booklets were sent to the parents. In the time from 1st contact from the ONS and the sending out of the baseline questionnaire, 450 families withdrew from TEDS, and 50 families had provided wrong or untraceable addresses. Consequently, baseline questionnaires were sent successfully to all but ~500 families (n=16 302).

The baseline questionnaire consisted of a single booklet addressed to the parent(s) or guardian(s) registered in the ONS database. Along with the questionnaire, a cover letter and TEDS information was sent along. Up to a maximum of 6 reminders over 11 months were sent to households that not had returned the baseline questionnaires. Overall 13488 families returned the baseline questionnaire, equivalent to a response rate of 87.2%.

1.1.1.2 Data collection in sub-cohort 3

The invitation to fill in the online food and drink preference questionnaire was posted and e-mailed to 3166 twin pairs at the start of the Easter holiday (April 2015). The invitation is shown in **Appendix B1.** Overall, 1305/3166 pairs (41% of cohort 3) completed the online questionnaire. 450 unpaired twins also provided data, 40 twins started but did not complete the entire survey.

Completion of the online food and drink preference questionnaire was rewarded with a £10 Flexecode voucher and entry into a prize draw to win a pair of iPad Minis. Entry into the prize draw was conditional on completion of the questionnaire by the co-twin, to encourage responses from complete twin pairs. Responses to each question were mandatory to complete the questionnaire. The procedures followed were in accordance with King's College London ethical standards on human experimentation and approval was obtained from the relevant committee on human subjects.

3.3 Measures

3.3.1 Socio-demographic information

Socio-demographic data for the sample was recorded in the baseline questionnaire (as described in the previous section).

3.3.1.1 Age and sex

Data on biological sex of the twins was parent-reported in the baseline questionnaire. Age of twins upon completion of the questionnaire used in this thesis was calculated from the date of birth, and the date (day/month/year) on which the questionnaire was completed.

3.3.1.2 Ethnicity

Parental ethnicity was used to assign ethnicity of the twins. In the baseline questionnaire, parents were instructed to select their ethnicity from four possible categories: 'Asian', 'Black', 'White', 'Mixed', or 'Other'. In cases where parents selected different ethnicity categories, twins were classed as 'Mixed'. Ethnicity categories were later dichotomized into 'White' or 'Other' due to the low numbers of participants in the non-'White' categories.

3.3.1.3 Socioeconomic status

Family socioeconomic status was coded as a continuous composite score, calculated from five different derived variables relating to parental educational qualifications, parental employment status and maternal age at birth of first child. Parental educational qualifications were coded from 1 to 8, with the lowest score corresponding to 'no qualification' and the maximum score corresponding to 'postgraduate education'. Parental employment was derived from various variables that asked about status, rank and supervisory tasks in their main job. Maternal age at birth of first child was calculated from the mothers' date of birth and the birth date of other siblings in the same family. Maternal age at birth ranged from 13 to 46 years of age. Composite SES scores were available for n=2725 families in sub-cohort 3. mean SES score was 0.35 (SD=0.96), with a range from -2.23 to 2.49. The higher the standardised SES score, the higher the socioeconomic statsus.

3.3.2 Anthropometric information

Body weight and height were self-reported by twins in the food and drink preference online questionnaire when they were 18-19 years of age. Weight was recorded using a choice of imperial (stone/pounds/ounces) or metric units (kilograms). Similarly, participants were asked to self-report height in imperial units (feet/inches) or metric units (centimetres/ metres). Height and weight measurements were converted to metric units to calculate body mass index (BMI). BMI was calculated by dividing an individual's self-reported weight (in kg) by the square of their self-reported height (in metres), to give a rough measure of adiposity (kg/m^2).

Although BMI has been criticized as an imperfect measure of adiposity as it doesn't distinguish between fat and lean tissue weight, it remains a useful index of adiposity for large population samples, at a low cost. BMI was used to categorise participants into four groups: Underweight (16-18.49 kg/m^2), Healthy weight (18.5 – 24.99 kg/m^2), Overweight (25 – 29.99 kg/m^2) and Obese ($\geq$30 kg/m^2) (WHO 1995). Study subjects (n=15) with implausible BMI values (<16 kg/m^2) were excluded from analyses when relationships between food and drink preferences with BMI were undertaken. BMI was also grouped into deciles by dividing the eligible sample into ten evenly-sized categories calculated from the observed range of eligible BMI values (16.06 – 59.81 kg/m^2). Self-reported height and weight has previously been validated as a reliable proxy measures for BMI in older adolescents (Brener et al. 2003; Goodman et al. 2000). A summary of socio-demographic characteristics for TEDS sub-cohort 3 is show in **Table 3.2** below.

Table 3.1 Demographic characteristics of the TEDS study sample (n=2865 individual adolescents)

Characteristic	Sample	
Sex [*n* (%)]		
M	1152	(40.2)
F	1713	(59.8)
Zygosity [*n* (%)]		
MZ[1]	1010	(35.3)
DZ[1]	1855	(64.7)
Age (SD)	19.1	(0.3)
BMI (SD)	22.3	(4.2)
Ethnicity [n (%)]		
White	2722	(94.9)
Other	143	(5.1)

[1] *Abbreviations: MZ=Monozygotic, DZ=Dizygotic*

3.3.3 Zygosity

Fundamental to the twin study methodology is accurate assignment of twin zygosity. MZ twins share 100% of their DNA whereas DZ pairs share on average 50% of their segregating genes. The twin method relies on resemblance between MZ and DZ pairs;

if resemblance between MZ pairs is substantially larger than that between DZ pairs, then genetic effects are assumed to influence variation for the observed characteristic. Misclassification of zygosity would therefore bias heritability estimates.

The gold standard for zygosity assignment is DNA testing (Hannelius et al. 2007; Jackson et al. 2001). However, this is a very costly method and not feasible for large population-based studies. Additionally, some study participants object to having their DNA sequenced. Questionnaires offer a reliable, acceptable, and more cost-effective alternative. Zygosity assignment based on phenotypic similarities which have a strong genetic basis and the extent of identity mix-up by others, has been shown to be highly reliable (95% accurate) when compared to genetic markers of zygosity (Price et al. 2000; Rasmussen & Johansson-Kark 2002). In TEDS, zygosity was assigned using a 20-item questionnaire in combination with a zygosity algorithm, full details of which are discussed in the following section. Zygosity assignment using DNA testing was also conducted for twin pairs where the zygosity algorithm was inconclusive.

3.3.3.1 Zygosity algorithm

A 20-item zygosity questionnaire was sent to parents at 1st contact, year 3, and year 4 of the study. A description of the 20 items can be found in **Appendix B2**. Opposite sex twin pairs were automatically classified as DZ. For each same-sex twin pair, the 20 items on the zygosity questionnaire were summed to create a difference score, with higher values indicating more differences between the twins. A zygosity score was calculated for each twin pair if a minimum of 10 items had been answered. The raw difference score was divided by the theoretical maximum score of 27 for each pair, which re-scaled the score to decimal values ranging from 0 to 1. This gives a 'zygosity index' score, whereby a higher score indicates greater differences between a twin pair. Pairs with a score of $\leq$ 0.64 were classified as MZs (i.e. smaller differences), pairs with scores of >0.70 were classified as DZs (i.e. higher differences). Pairs with zygosity scores between 0.64 and 0.70 were unclassified and DNA was used to assign zygosity unless parents reported any of the following observations: clear differences in eye or hair colour, hair texture differences, or did not perceive their twins to look much alike at all. In addition, pairs were classified as MZs if they were reported to be 'as alike as two peas in a pod'.

3.3.4 Food and drink preferences

Food and drink preferences were assessed using an online questionnaire, the development of which is described fully in **Study 1** (**Chapter 4**). This questionnaire collected self-reported liking of 69 common food items and 9 beverage types on a 5-

point Likert scale. For each food item, twins were asked “How much do you like...”, with response options ranging from ‘like a lot’ to ‘dislike a lot’. A higher score was indicative of higher liking. Participants were instructed to indicate any items that were unfamiliar or if they had no recollection of ever tasting the item before; these items were coded as missing. Food items were grouped into categories based on a principal components analysis. A detailed description of this statistical procedure is also included in Study 1.

3.3.5 Dietary patterns, self-rated healthfulness of diet, and food allergies

Data on dietary restriction and important food allergies were also collected in the food and drink preference questionnaire. Self-perceived healthiness of current diet was measured with the question “In general, how healthy would you say your current diet is?”, with response options ranging from ‘very healthy’, ‘somewhat healthy’, ‘neither particularly healthy or unhealthy’, ‘somewhat unhealthy’ to ‘very unhealthy’. Participants were instructed to indicate their dietary pattern from the following list: ‘vegan’, ‘vegetarian’, ‘pescetarian (no meat, but eat fish and/or shellfish)’ or ‘none of the above’. Participants were further asked to declare whether they were allergic to a list of 10 common food allergens (e.g. peanuts, shellfish, dairy). A comprehensive overview of these measures is available in **Study 1**.

3.4 Statistical analysis

This thesis uses a variety of statistical techniques to investigate the aetiology of food and drink preferences and relationships with adiposity in the TEDS sample (**Study 1, 2** and **3**). Broadly, these quantitative approaches can be split into two categories: (a) twin analyses to investigate the aetiology of food and drink preferences and (b) between- and within-family analyses to examine the association between food and drink preferences and BMI. The food and drink questionnaire data collected in the TEDS sample was analysed using principal components analysis (PCA) to establish the structure of food preferences, with a full description of the method in **Chapter 1**.

3.4.1 Twin analyses

The twin design of TEDS provides a powerful strategy to understand the genetic and environmental influence on variation in food and drink preferences in a specific population at a specific point in time. The following section will briefly summarize the underlying logic and assumptions of the twin method, before discussing details of the different modelling methods that were used in this thesis.

3.4.2 The twin method

The twin method is often described as the 'perfect natural experiment' to disentangle the relative contribution of nature and nurture to variation in a measurable trait. The basis of the twin method is to compare the degree of resemblance between identical or monozygotic (MZ) pairs who share 100% of their genes, with that between non-identical or dizygotic (DZ) pairs who share approximately 50% of their segregating genes. The more similar MZ pairs are for variation in a trait relative to DZ pairs, the stronger the genetic contribution to the trait. This inference is based on the assumption that MZ twins are twice as similar genetically than DZs (Plomin et al. 2013). The key thing is that both types of twins share their environments to a very similar extent – that's why it can be assumed that any difference between them is attributable to genetic differences between them (because the extent of shared environmental influence is assumed to be the same).

The statistic derived to estimate the genetic contribution to variation in a trait is called 'heritability' (A; additive genetic influences), and can be thought of as an index of the genetic effect size. In a broad sense, heritability refers to the degree to which a trait is genetically determined, and encompasses all additive and non-additive genetic variance. Additive genetic variance includes the inter-individual variability that is attributable to genetic variants that influence the same trait and their effects works together on the phenotype. On the other hand, non-additive genetic variance comprises dominant genetic factors (genetic variants which mask the contribution of a recessive genetic variant) and epistatic genetic factors (genetic variants at one position on the chromosome masking the expression of a separate genetic variant on a different position of the chromosome). In the classic twin model, heritability only considers additive genetic variance (Boomsma et al. 2002). The remaining variance is partitioned between: shared environmental influences (C; any factors that make two twins in a pair more alike beyond the effects of genetics, such as the home, shared family practices and schooling experiences); and non-shared environmental factors (E; any experiences or influences that account for differences between twin pairs such as having different friends or bouts of illness experienced by one twin only) which includes random measurement error.

Intraclass-correlation coefficients indicate the magnitude of resemblance of co-twins within a pair and values range from -1 to 1 (but typically range from 0 to 1 for twin pairs because it is highly unusual to observe negative values). The closer the ICC value to 1, the more alike twins are for a trait. Broad patterns in r_{MZ}/r_{DZ} intra-class correlations can be used to infer heritability of a trait. Additive genetic factors are implicated in influencing variation in a trait if r_{MZ} is greater than r_{DZ}. If $r_{MZ}*2 \geq r_{DZ}$ then this is suggestive of substantial non-additive (dominant) genetic influences. On the other hand, if $r_{MZ} \leq 2*r_{DZ}$, shared-environmental influences are likely to be contributing to variation in a trait. Low or near-zero intra-class correlations for both MZ and DZ twin pairs suggest a strong non-shared environmental influence on individual differences in a trait.

Exploiting differences in within-pair correlations between MZ and DZ twin pairs (r_{MZ} and r_{DZ}), Falconer's formula can be used to estimate genetic and environmental influence on a phenotype based on the pattern of the twin correlations:

$$A = 2\,(r_{MZ} - r_{DZ})$$
$$E = 1 - r_{MZ}$$
$$C = r_{MZ} - A$$

Equation 1 Falconer's formula

A = heritability, C = shared environmental influence, E = non-shared environmental influence, r_{MZ} = intraclass correlation for MZ twins, r_{DZ} = intraclass correlation for DZ twins

Fundamental to the classical twin study are three main assumptions (Rijsdijk & Sham 2002). These three main assumptions are:

[1] The equal environments assumption: MZ and DZ twin pairs experience roughly equal environments, and share their environments to the same degree.

[2] Non-assortative mating: Mating occurs randomly in a population, independent of familial relatedness or social likenesses which may increase genetic similarity between mates. For instance, if mates select one another on the basis of a trait that is strongly influenced by genetics (e.g. IQ or body weight) then they themselves share genes in common, and their off-spring (who are not MZs) will therefore be more than 50% similar. If DZs are more than 50% genetically similar, the difference between MZs and DZs will be smaller for genetically-influenced traits, and heritability will be lower than expected (Fisher 1919).

[3] Twins are representative of the wider population (e.g. singletons) in terms of the trait or behaviour under consideration. This assumption is necessary to ensure that results from twins are generalizable to the wider (target) population.

Incorrect estimates of genetic and shared-environmental influences on individual variation in a trait arise if these assumptions are not met. There is some controversy surrounding whether these assumptions are met in studies of twins. The validity of the equal environments assumption is still particularly contested (Joseph 2013; Eaves et al. 2003). This assumption is considered the most basic assumption of twin models, and states that both MZ and DZ twin pairs experience the same environmental conditions. Because heritability estimates from twin studies are determined by the magnitude of the difference in within-pair resemblance between MZ and DZ twin pairs, heritability estimates may be inflated if MZ co-twins share their environment to a greater degree in a way that makes them more similar for the trait in question (Horwitz et al. 2003). For instance, MZ twins tend to look more similar, and some research has suggested that MZ twin pairs are treated more alike due to this physical resemblance (e.g. parents dress their identical twins the same, and MZ twins may be more likely to be placed in the same school class, etc) (Loehlin & Nichols 1976). Also, and perhaps because of socialization of MZ twins to be more alike compared to DZs, MZ may spend more time together in childhood (shared friends or playtime) and consequently may maintain higher contact frequency later in life (Määttä et al. 2016). Dalgard et al (1976) also suggested that on a more psychological level, MZ twins may experience greater “identity closeness” (Dalgard & Kringlen 1976). A revised definition of the equal environments assumption includes the specification that the environmental experiences for which the twin pairs types should not differ must be relevant to the aetiology of the trait in question (Kendler & Baker 2007). Recently, a review and pooled re-analysis of 16 twin studies concluded that the equal environments assumption holds true for most outcomes (i.e. for 31/32 outcomes – neuroticism being the exception) and that violation would only result in overestimation of heritability by around 10% (Felson 2014).

Nevertheless, classical twin studies provide the opportunity to establish genetic influence on population variance for a trait, and can guide future research to establish the source of this variation (Bouchard 2004). With recent technological advances and successes in the molecular genetic field, the value of twin studies (or quantitative genetic studies in general) lies in the fact that these studies also focus on identifying the sources of environmental variation, an area of research still largely neglected in genome-wide association studies (GWAS) (Haworth & Plomin 2010). In addition, twin studies estimate the cumulative effect of genetic influence regardless of the number of genes involved or the size of each of their separate effects. Because twin studies also provide information about the relative importance of shared versus non-shared environmental influence, they can help to guide researchers in relation to where best to target interventions. If there is high shared environmental influence, the home and family environment is probably important, but if there is high non-shared environmental influence, the wider environment might be a better target for modification. (Haworth & Plomin 2010). This knowledge is useful as it provides usable insights to develop targeted health behaviour modification intervention programs. Along with this, it also provides the justification to intervene, despite substantial heritability of disease risk.

Estimations of the aetiology of a trait from Falconer's formula provide only an initial indication of the pattern of influence. Maximum likelihood structural equation modelling is a much more sophisticated approach the provides robust estimates of A, C and E with 95% confidence intervals and goodness-of-fit statistics. However, the twin correlations always provide an important 'sense check' for the estimates derived from the more complex models, and both are important to make sense of findings from twin studies.

3.4.3 Maximum Likelihood Structural Equation Modelling of genetic and environmental correlations

Maximum Likelihood Structural Equation Modelling (MLSEM) estimates A, C and E based on prespecified covariation structures between MZ and DZ twins based on the key underlying assumptions explained in the previous section – the extent of their genetic relatedness and shared environment. MZ twins share 100% of their genetic material but DZ twins on average only share half of their genes. In line with this, the genetic correlation coefficient for MZ twins is therefore constrained to 1 whereas it is fixed at 0.5 for DZs. Because it is assumed that both types of twin pairs share their environment to the same extent, the shared environmental correlation is set at 1 for *both* MZ and DZ twins. Non-shared environmental factors contribute to differences between twins within a pair, thus the latent factor E is not correlated between twins.

Path diagrams are commonly used to display these defined correlation coefficients between twins, and the effects of the A, C and E on the measured trait, as shown in **Figure 3.3.** The latent factors are represented by circles, and the trait is shown as a rectangle. The double-headed arrows represent the genetic and shared environment correlation coefficients between the two different types of twins.

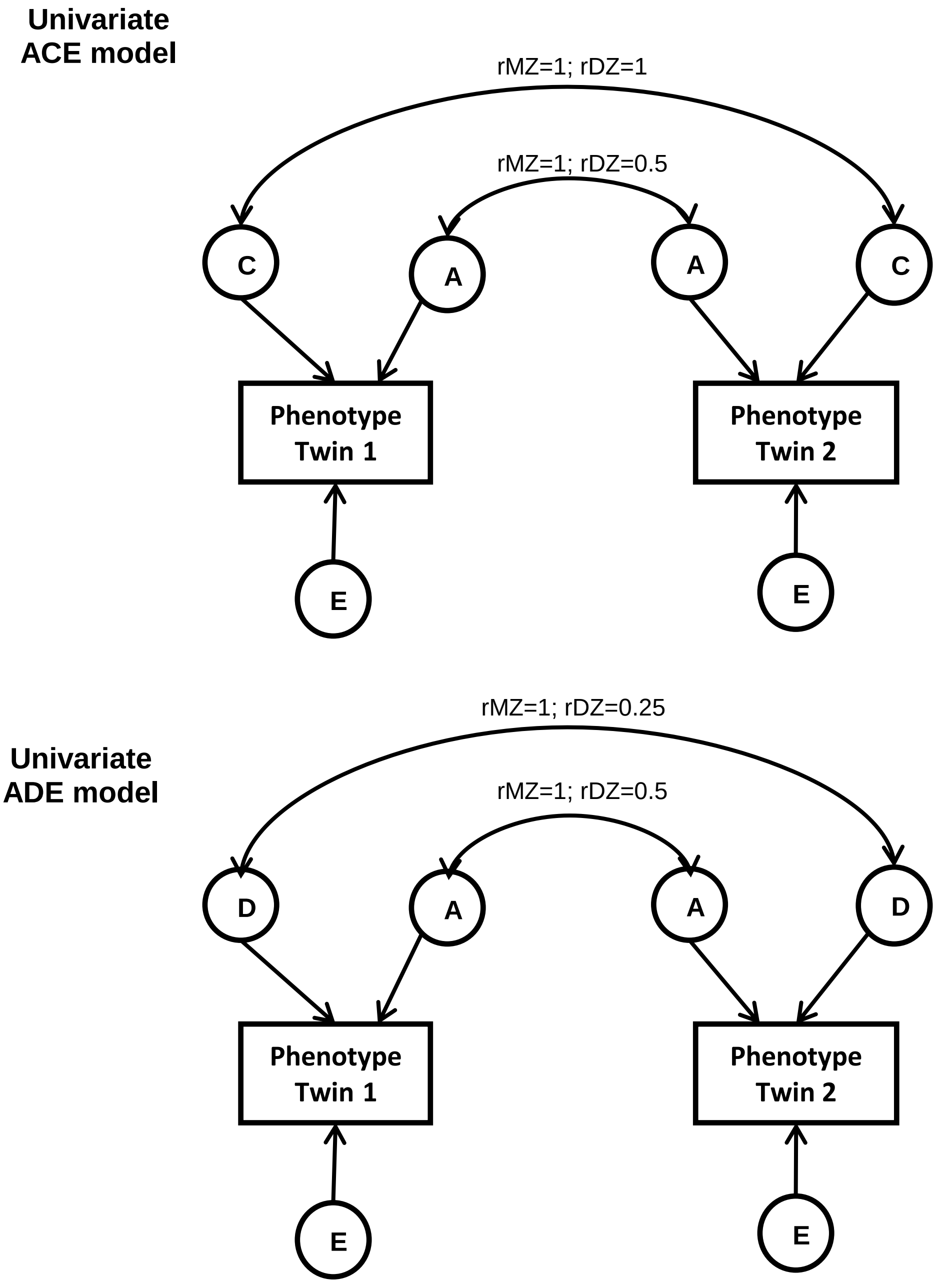


Figure 3.3 Path diagram representing the relationship between latent factors in the classic twin model for monozygotic (MZ) and dizygotic (DZ) twin pairs

The full ACE and ADE univariate twin models show the aetiological contributors to a measured phenotype, represented by the rectangular boxes ('scores'). The circles indicate the latent factors: additive genetic effects (A), non-additive genetic effects (D), shared environmental effects (C) and non-shared environmental effects (E). The straight single-headed arrows reflect standardised causal pathways with the variance explained by each latent factor (a, d, c, e). The curved double-headed arrows show the correlations between the latent factors for twin 1 and twin 2. The zygosity of the twin pair defines these correlations. MZ twins share all their genetic factors (r_A=1.0) whereas DZ twins only share half of their genetic factors (r_A=0.5). Both types of twins share all of their shared environmental factors (r_C=1.0). Non-shared environmental factors are not correlated between co-twins (r_E=0). In an ADE model, paths for dominant genetic factors (D – all non-additive genetic effects) are modelled instead of shared environmental factors (C). Dominant genetic factors are set at 1.0 for MZ twins and 0.25 for DZ pairs.

The sum of all pathways (for A and C) between twin 1 and twin 2 equals the total covariance of the trait.

Maximum Likelihood Structural Equation Modelling (MLSEM) is the standard methodology to analyse continuous twin data to estimate A, C and E). It uses variance and covariance between twins to estimate the latent factors, which are then represented as path diagrams. The matrices of the expected variance and covariance of a trait for MZs and DZs can be written as:

$$\text{Covariance}_{MZ} = \begin{bmatrix} \partial_A^2 + \partial_C^2 + \partial_E^2 & \partial_A^2 + \partial_C^2 \\ \partial_A^2 + \partial_C^2 & \partial_A^2 + \partial_C^2 + \partial_E^2 \end{bmatrix}$$

$$\text{Covariance}_{DZ} = \begin{bmatrix} \partial_A^2 + \partial_C^2 + \partial_E^2 & 0.5\,\partial_A^2 + \partial_C^2 \\ 0.5\,\partial_A^2 + \partial_C^2 & \partial_A^2 + \partial_C^2 + \partial_E^2 \end{bmatrix}$$

MLSEM provides reliable estimates of A, C, and E and corresponding measurement error (i.e. 95% confidence intervals), as well as goodness-of-fit statistics. MLSEM for this thesis was undertaken using the software package OpenMx (Boker et al. 2011) in R (version 3.3.1) (R Core Team 2015). In brief, OpenMx uses maximum likelihood to estimate the latent variables that fit the observed variance-covariance structure of the twin data best. it is standard practice to carry out analyses on scores residualised for age and sex effects because all twins share their age exactly (and sex for same-sex twins), and these factors can therefore inflate the shared environment effect (McGue & Bouchard 1984). To begin with, a saturated model is fitted to the data with no parameter constraints, estimating only means covariances and variances for MZ and DZ separately. The saturated model provides reference fit statistics, against which a full ACE model and three submodels can be compared. To identify the most parsimonious model, sub-models consecutively dropping the A and C parameters (E is never dropped from the model because it includes measurement error) are nested within the full ACE model. Selection of the most parsimonious model is based on goodness-of-fit statistics: the Likelihood Ratio Test (LRT) assessed using chi-square (χ^2) and Akaike Information Criterion (AIC) (Akaike 1987). For the LRT, dropping parameters penalizes the goodness-of-fit for the model, and the best fitting model with increased explanatory power is indicated with the lowest AIC score. On the other hand, the AIC favours the model which explains the greatest proportion of data, and rewards goodness-of-fit for models with the lowest number of parameters. Comparing the AIC of two models, a difference >2 provides some support for selecting one model over the other. Overall, the most parsimonious solution is identified by the lowest absolute value of the AIC and smallest $\Delta\chi^2$ (Burnham & Anderson 2003). The AIC is also used to compare goodness of fit of non-nested models such as ACE versus ADE. As before, the model with the absolute lowest AIC value is selected as the most parsimonious solution to explain the structure of the underlying twin data (Akaike 1987).

OpenMx allows for data from incomplete twin pairs to be included in analyses. By using full information maximum likelihood (FIML), the software can compute unbiased parameter estimates and standard error estimates for each individual in a raw dataset, reliant on data to be missing at random. Because model fit is derived from the sum of fit functions across each individual in a raw dataset, FIML is a pragmatic approach for all available observations to be included in analysis (Enders 2006).

3.4.3.1 Univariate ADE models

The classic ACE twin model is restricted to capturing ‘narrow’ heritability, i.e. only additive genetic influences are included in latent factor A. However, genetic effects can also be transmitted in other manners. For instance, non-additive genetic influences, (or ‘dominant’ genetic influences [D]) represent interactions between alleles at the same locus such as genetic dominance (a type of inheritance/expression), gene-gene interactions, and gene-environment interactions (Rijsdijk & Sham 2002). ICCs greater than twice the DZ ICCs (rMZ > 2 *rDZ) for a phenotype suggest non-additive genetic effects. A limitation of the classic twin model is that it can only estimate three parameters at a time, i.e. it is unable to model shared environmental effects (C) as well as dominant genetic effects (D) in the same model (Chen et al. 2015). Dominance effects can only therefore be estimated in a model which includes D instead of C. The fundamental difference between the ACE and ADE models is the twin correlation is defined as 1 for MZ twins while it is set to 0.25 for DZ twins in the ADE model, while in the ACE model, this is set to 1 for MZ twins and 0.5 for DZ twins. This necessarily changes the way variance components are decomposed to allow for the estimation of possible non-additive genetic influences.

MLSEM model-fitting for ADE models is analogous to the process for ACE models (explained in detail in the previous section).

3.4.3.2 Modelling dichotomous rather than continuous data

The liability threshold model is used to analyse ordinal or binary outcome variables, rather than continuous data. It can be used as well when data are skewed, and variables are therefore dichotomized. This model makes the assumption that the phenotype results from an underlying continuous variable which follows a normal distribution. Individuals are assumed to be ‘affected’ or express a specific phenotype when their position on the liability distribution crosses a defined threshold value. Individuals are categorised into ‘affected’ vs ‘unaffected’ in accordance to their position on the liability threshold distribution (Neale et al. 1994).

In this thesis, mean food and drink preference scores were generally high (the main outcome variables), resulting in negatively skewed distributions for these preference scales. Because scores for drink preferences were skewed to a greater extent than food preferences, continuous drink preference scores were modelled as dichotomous data. Drink preference scores were summed and dichotomized by the median of the scale into ‘high likers’ vs ‘low likers’. For dichotomous data, tetrachoric correlation coefficients (TTC’s) were used to calculate phenotypic concordance rates for MZ and DZ pairs instead of intraclass correlations. MLSEM uses the ratio of affected and unaffected twins, split by MZ and DZ twin pairs, to estimate A, C and E (Rijsdijk & Sham 2002).

3.4.3.3 Sex-limitation models

MLSEM can also be used to assess potential sex differences in genetic and environmental influence on a trait. There are three different types of sex differences: (i) ‘*qualitative* sex-differences suggest that the genes and environmental influences on a trait are *different* for males and females (i.e. *different* genes are involved); (ii) ‘*quantitative*’ sex differences indicate that the magnitude of A, C, E are different for males and females (i.e. the genes and environmental influences are *the same* but they have different effects sizes for males and females); (iii) variance differences can exist in the phenotype for males and females, even though the parameter estimates (A, C and E) are the same across the sexes (Neale et al. 1992). It is important to test for these effects as a mixed-sex twin sample may be disguising important insights on the aetiological architecture of a trait if it is only investigated in the mixed-sex sample (Chen et al. 2015). For example, it is possible for a trait to be 100% heritable for males, and not at all heritable for females, which would indicate an average heritability of 50% if both sexes are combined.

To model sex differences the twin sample is spilt into: same-sex pairs (MZ males (MZM), DZ males (DZM), MZ females (MZF), DZ females (DZF)); and opposite sex DZ pairs (DZos). Intraclass correlations for each subset of twin pairs provide an initial indication of sex differences. Qualitative sex differences are suggested if DZss >

rDZos. Quantitative sex differences are suggested of the ratio of rMZM/rDZM is substantially different from MZF/DZF. A full ACE sex-limitation model can then be applied to test nested MLSEM models to identify if a sex-limitation model provides the most parsimonious explanation of the underlying twin data. The model against which subsequent models is tested allows for *both* qualitative, quantitative and variance differences; a sub-model constrains qualitative differences and allows only quantitative differences; then another model constrains qualitative and quantitative differences and allows only variance differences; then the final model is a null model that constrains everything to the same across the two sexes (a standard univariate ACE model).

3.4.3.4 Statistical significance and power

The statistical power of a univariate twin model is a function of the size of the specific parameters under examination (e.g. heritability), overall sample size, the ratio of MZ/DZ twins, the type of data being analysed (continuous vs. categorical), the number of variables being considered (univariate vs. multivariate), and the probability level (α) (Neale et al. 1992). While the estimation of statistical power for a twin study is equivalent to any other procedure, it must also be considered that power to detect a specific variance component (e.g. A) in a twin study is dependent of the magnitude of the other variance components in the model (e.g. C or E).

Power analyses for a univariate twin model using the TEDS data were undertaken on simulation data by Verhulst (2017). Food and drink preference data from TEDS were available for 1243 complete twin pairs and 379 unpaired co-twins. Graphs summarising the statistical power of twin modelling scenarios, varying in the magnitude of heritability and effect size of the shared environment, as well as the ratio of MZ/DZ twin pairs are available in **Appendix B3.** Heritability and environmental effect size values from a previous twin study on food and drink preferences in a similar sized sample of 3-year old twins (n=2686) were used to approximate power in the TEDS dataset (n=2865) to detect underlying variance components (Fildes, van Jaarsveld, Llewellyn, et al. 2014). The smallest genetic effect was seen for ‘Dairy’ (A=0.27) and the smallest effect of the shared environment was seen for ‘Vegetables’ (C=0.35). Based on these estimates, a sample size of $\geq$1000 twin pairs has good power (>90% to detect effect size parameters under all hypothetical scenarios described (Verhulst 2017). Power is maximized when shared environmental influences are high (C$\geq$0.50).
In addition, the TEDS sample consisted of 1010 MZs and 1855 DZs. This approximately equates to a 1:2 ratio of MZ:DZs. Based on these parameters, the TEDS sample was powered at >90% to detect significant genetic and environmental variance

components with a sample of over 100 twin pairs with a 1:2 MZ:DZ ratio (see **Appendix B3 (b)**).

3.4.4 Analyses to investigate relationships between food and drink preferences with adiposity

I used both between- and within-family analyses of TEDS data to examine associations between food preferences and BMI. Between-family analyses included both twins from each pair, so Complex Samples General Linear Models (CSGLMs) were used which take into account the clustering of twins within a family. The within-family analyses modelled differences between identical twin pairs in BMI and food preferences, which takes account of confounding in the relationship between shared environmental factors and genetic influences on each trait. The between- and within-family analyses are summarised below. Specific details on each statistical procedure can be found within each relevant chapter.

3.4.5 Between-family analyses

The non-conventional sampling methodology of a twin study differs from a typical simple random sample because twins cluster within families – i.e. they are not independent from one another and the measured traits of interest (e.g. food preferences and BMI) tend to be correlated in twin pairs. Clustering of twins reduces the sample standard error, therefore inflating the probability if making of a type I error (rejecting the null hypothesis when the null hypothesis is true).

Complex Samples General Linear Modelling (CSGLM) was used for between-family analyses because it adjusts for clustering of twins in families. The strength of the association between food preferences and BMI is represented by the unstandardised β-coefficient, indexing the gradient of the regression line fitted between the predictor and outcome variable. Essentially, β gives the predicted change in the outcome variable for each one unit increase in the predictor. CSGLM adjusts the standard error of the β-coefficient to account for the paired structure of the twin data (Carlin et al. 2005). A higher value of β is indicative of a stronger association. The effect size is estimated by R^2, equivalent to the proportion of variance in the outcome variable that is explained by the predictor variable (or cumulative effect of multiple predictor variables entered in the model simultaneously). Five assumptions must be met to estimate the association between variables accurately and reliably (Field 2013) (details of the empirical tests of these assumptions are reported in **Chapter 6**):

[1] Linearity of the relationships between the predictor and outcome variables: this was assessed in scatterplots.

[2] Independent errors of the residuals: the Durbin-Watson test was used to test for the presence of autocorrelation between errors of the residuals. Test statistic values range from 0-4, with a value of 2 signifying no autocorrelation. Test statistics >2 indicate negative correlations between adjacent residuals, whilst test statistics <2 are indicative of positive autocorrelations (Durbin & Watson, 1951).

[3] Homoscedasticity (the variance of the residuals needs to be constant for each level of the dependent variable): this was assessed using scatterplots of the residuals.

[4] Normality of the residuals: this was assessed visually using Q-Q plots of the residuals.

[5] Multicollinearity (the predictor variables should not correlate too highly (r<.8): this was assessed using Pearson's correlation coefficients between predictor variables (Field 2013). The absence of multicollinearity was further assessed via Variance Inflation Factors (VIFS), with a VIF <10 for predictor variables confirming the absence of multicollinearity.

3.4.6 Within-family analyses

Based on the core assumptions of the classical twin study, the genetically sensitive nature of twin data allows for several elegant study designs not possible in samples of unrelated individuals. One of these options is the discordant twin design, which is an example of the utility of a twin study to explore the environmental basis of intra-individual differences for a trait (Bouchard & Propoping 1993). This design was used in Study 3 of this thesis.

Even for very heritable traits, twins can be discordant for a phenotype. Discordant siblings are useful to study the relationship between an exposure and an outcome, controlling for confounding by shared-environmental factors possibly implicated in shaping. Comparison for a risk factor between discordant MZ and DZ co-twins' controls for C effects and partially for A effects (as DZ twins only share 50% of their genes). Restriction of within-pair comparisons for discordant MZ twins further controls for confounding of C and A effects. This design is ideal to rule out a genetic contribution to the observed difference between co-twins. Importantly, within-pair comparison cannot control for confounding due to the non-shared environment (E). Consequently, comparison of rare monozygotic (MZ) twin pairs discordant for body mass index provides a unique opportunity to identify non-shared environmental influences on adiposity. Within-MZ pair analysis provides the greatest control for confounding since these twins share not only identical genetic background, but also early life events and the family environment (Sahu & Prasuna 2016).

In Study 3 of this thesis, patterns of food and drink preferences were compared between adiposity-discordant MZ twins to describe dietary preferences that accompany BMI-discordance. The strength of this approach lies in its ability to control for all genetic and environmental confounding shared by MZ co-twins. This design thus allowed to identify mechanisms (via E) that play a role in shaping bodyweight variability, independent of genetic or shared-environmental confounding (McGue et al. 2010; Zwijnenburg et al. 2010).

3.4.7 Statistical significance and power

Alpha is most commonly set at .05, but the large sample size of TEDS (n=2865) increases statistical power which means that adjustment of the p-value is necessary to reduce the risk of type I errors. For the between-family analyses in TEDS the alpha level was therefore set at .01; this lower p-value threshold also adjusts for multiple testing. For the within-family analysis conducted in the TEDS sub-sample in **Study 3b** (n=77 pairs), α was kept at the conventional threshold of 0.05 to reflect the much smaller sample size and reduced power. To estimate the magnitude of the associations or the observed differences, independent of sample size, effect sizes were assessed with correlation coefficients, Cohen's d (for t-tests), eta squared (for ANOVA), and R^2 for CSGLM. Power calculations were undertaken in G*Power (Version 3.1.9.2; Softepdia).

3.4.7.1 Power for Complex Samples General Linear Models

A CSGLM model with one = predictor and a sample size of ~2865 was powered at 99% power to detect an R^2 of 0.001 (equivalent to 1% in the variability of the dependent variable).

3.4.7.2 Power for within-family analyses

Based on data from 77 twin pairs, the co-twin control design was powered >80% to detect a medium effect size (Cohen's d=0.5) at a significance level of α=0.05. Power calculations for between-pair analyses (n=455 MZ pairs) indicated that with one predictor variable, an unstandardized regression coefficient of β=0.15 could be detected at α=0.05 with a power >80%.

3.5 Summary

TEDS is a large population-based representative twin birth cohort which provides the opportunity to disentangle the relative importance of genetic- and environmental influences on food and drink preferences in adolescence. Additionally, the collection of anthropometric information within this genetically sensitive study design enables the application of a variety of approaches to explore the role of food and drink preferences in adiposity variation during late adolescence.

As mentioned at the start of this chapter, in addition to the TEDS sample used for **Studies 1, 2** and **3**, an independent sample of young adults was recruited for the fourth and last study of this thesis. **Study 4** is a randomised controlled pilot trial to test the feasibility and effectiveness of a sweetness preference modification intervention. The development of the study protocol and materials for **Study 4** was informed by the findings from the three preceding studies. Details of the sample, measures and statistical analysis for **Study 4** are described in **Chapter 7**.

Chapter 4.

Study 1 - Development of a food and drink preference questionnaire for adolescents and adults[I]

4.1 Background

The literature review in **Chapter 1** emphasizes the importance of food and drink preferences as key determinants of dietary intake in adolescents (Drewnowski et al. 1999; Birch & Fisher 1998; Woodward et al. 1996; Perry et al. 1990). In childhood, food preferences are influenced importantly by environmental factors beyond the individual's control; but as children mature, they progressively encounter more eating moments outside of the home environment and begin to expand their food preferences independently. Adolescence is a critical phase in an individual's development, characterised by many physiological and psychological changes, as young people make the transition into independent adults.

Most research into food preferences to date has focused on either young children or adult populations. Food and drink likes and dislikes in adolescence are poorly researched even though this is recognized as a vital developmental stage (Perry & Murray 1982).

Because food and drink preferences drive decisions surrounding ingestive behaviour, it is crucial for research that these characteristics are reliably and accurately measured. The measurement of food preferences can take many different approaches, ranging from the examination of general dietary patterns, macro nutrients, food groupings and single food items, down to the level of preferred intensities of the five basic tastes (Newby & Tucker 2004). Variation in food preferences has repeatedly been shown to be best explained by organising individual food item scores into categories (Domel et al. 1993; Drewnowski & Hann 1999). Focusing on food groups rather than individual items makes it easier to inform simple recommendations that can be incorporated into daily life (Hasselbalch 2010). In addition, categorization of individual food items into food groups makes it easier to establish the broad influences on food preferences rather than item specific affinities or aversions (Newby & Tucker 2004).

[I] The questionnaire is available as part of the *Eating Behaviour Questionnaires* section on the UCL Department of Behavioural Sciences website: http://www.ucl.ac.uk/iehc/research/behavioural-science-health/resources/questionnaires/eating-behaviour-questionnaires
A copy of this publicly available questionnaire is shown in **Appendix G.**

Two previous studies have designed parent-report measures of food preferences for young children (Fildes, van Jaarsveld, Llewellyn, et al. 2014; Wardle, Sanderson, et al. 2001). However, preferences can change over time as children mature into adolescence; and currently there is not a valid and reliable food and drink preference questionnaire for adolescents. A prerequisite to studying genetic and environmental influences on variation in liking for different food groups and drink types, and their relationships with adiposity during this important developmental stage, is a valid and age-appropriate questionnaire.

4.2 Study aims

The aim of this study was to adapt and update existing parent-report questionnaires of young children's food and drink preferences, to create a self-report measure of variation in food and drink preferences of older adolescents.

4.3 Methods

The questionnaire development was conducted in three stages: (1) a comprehensive list of food and drink items from previous food and drink preference questionnaires was compiled, updated and tailored to ensure items were relevant for adolescents in the current food environment, (2) a test-retest pilot study was undertaken to assess the suitability of the items and the acceptability of the online questionnaire, and (3) the final questionnaire was distributed to a large UK adolescent sample in order to establish the factor structure and internal reliability of the measure.

4.3.1 Sample

Participants for the core study were 3290 individual twins from sub-cohort 3 of the Twins Early Development Study (TEDS), described in detail in **Chapter 3**. Data were excluded from the twins with serious medical- or perinatal problems, those that had skipped the food and drink preference questionnaire section leaving a final sample of 2865 individuals.

4.3.2 Questionnaire development

The food items selected for the questionnaire were primarily based on the 94 items in a previous parent-report questionnaire used in a subsample of 4-5 year old twins from TEDS (Wardle, Sanderson, et al. 2001), and a subsequent 'modernised' version used for a heritability study of food and drink preferences in children in 2014 (Fildes, van Jaarsveld, Llewellyn, et al. 2014). The 94 items included in Wardle and colleagues' food preference questionnaire had initially been derived from two food frequency questionnaires (FFQs). The first was a validated FFQ of 35 diverse single food items commonly consumed by British school children (Hammond et al. 1993). The other was the 'European Prospective Investigation into Cancer and Nutrition (EPIC) FFQ' (Bingham et al. 1997), the British equivalent of a FFQ used in the US Nurses' Health Study (Willett et al. 1983). This US FFQ was designed to represent the major sources of the 16 main nutrients in the average US diet, and measured consumption frequency of 99 food items. For epidemiological research, FFQs have been designed to measure habitual dietary intake which highly correlates with typical daily nutrient consumption. Established and validated FFQs have provided a useful template for the design of food preference questionnaires, because they include a comprehensive list of commonly consumed food items (Hu et al. 1999) .

From this initial list of 94 food items, some composite main meal foods that did not measure specific individual food preferences were removed. We considered items to be composite main meal foods if they were considerably processed, or items that contained multiple ingredients (e.g. quiche, pizza, meat pie, vegeburger, shepherd's pie). A number of more recent and age-appropriate items were added, including hummus, tinned tuna, peanut butter and smoked salmon. Items consumed primarily by younger children were excluded: e.g. jelly, ice lollies, and jelly babies. Given some notable research on liking for Marmite (YouGov UK 2011) and coriander (Eriksson et al. 2012), these two items were also added. Lastly, questions regarding more general liking for sweet, salty or spicy tastes were included (e.g. 'How frequently do you add salt to your food?'). Drink items were added as a separate section, after the food items. In total nine different drink items were added, representing the most commonly consumed drinks among 18-19-year-olds in the UK: non-diet fizzy drinks, diet fizzy drinks, orange juice, fruit squash (e.g. orange squash), milk, tea, coffee, beer and wine. The 69 food items and 9 drink items included on the complete final food and drink preference questionnaire are shown in **Appendix C1**.

Special rules applied for the completion of this questionnaire due to the online data collection process. Firstly, completion of an online consent form after the initial login was mandatory. Overall the questionnaire required around 10 minutes to complete and for this reason twins were encouraged to complete the survey in one go. Nevertheless, should the survey be interrupted mid-way, it was possible for participants to resume the survey by logging in to the online portal at a later date.

Completion of each question was compulsory in order to progress through the survey; this ensured no missing data. Twins were instructed to select the 'Not applicable' option for any food items they were unfamiliar with. Branched questions were used to assess liking for tea, coffee and wine. Preference ratings for these items were only sought if the initial question 'Do you drink tea/coffee/wine' was answered with a 'Yes'.

4.3.3 Food and drink preference items

The final food preference questionnaire listed 69 food items which the twins were asked to rate their liking for. Participants were instructed using the following: ‘Briefly read the following list of food items and tick the box which most accurately reflects how much (on average) you like the specific item (not necessarily how much you actually consume)’. The following five response options were provided for each item of food: ‘dislike a lot’, ‘dislike a little’, ‘neither like nor dislike’, ‘like a little’, ‘like a lot’. Participants were instructed to select ‘not applicable’ if they were not familiar with, or couldn’t remember having tried a food item. Liking of nine drink items was measured on the same 5-point Likert scale used to record food preferences. Responses were coded with values ranging from 1 to 5, with a higher score indicating a higher preference for a food item. “Not applicable” was coded as missing.

4.3.4 Test-retest pilot study

A two-week test-retest pilot study was undertaken to assess the suitability of the food and drink preference items, the reliability of self-reported food and drink preference scores in adolescents, and to test the acceptability of the online questionnaire. Feedback on any aspect of the questionnaire was collected at the point of completion in an open response text box. Participants in the test-retest pilot were a sample of the twins’ siblings (n=205); they received a £10 electronic shopping voucher for completing the questionnaire the first time; completion of the *retest* questionnaire was compensated with an additional £5 online shopping voucher.

4.3.5 Measures

The food and drink preference questionnaire was sent out and completed by the twins in April 2015. In this questionnaire participants were also asked about any important food allergies or dietary requirements that may influence their habitual intake and liking of certain foods, using the question: ‘Do you identify as any of the following?’ Response options included: ‘vegan’, ‘vegetarian’, ‘pescetarian (no meat, but eat fish and/or shellfish)’ or ‘none of the above’. A list of the ten most common food allergens in the UK was provided and the twins were asked to indicate all of the items to which they were allergic (peanuts, tree nuts, sesame, dairy, soya, shellfish, fish, egg, gluten/wheat, celery or mustard). The full list of food sensitivities can be found in **Appendix C1.** An open response box was included to allow participants to report any food allergies not listed.

4.3.6 Statistical analysis

4.3.6.1 Factor structure of the food preference questionnaire

Investigations into possible categories of food preferences may be based on a previous established model or theoretical structure; these investigations can use confirmatory factor analysis (CFA) which tests whether observed data fits a defined model (Syms 2008). However, because of the substantial revisions to the food and drink preference questionnaire and the higher age of the current sample compared to those used to develop the earlier food and drink preference questionnaires, a more exploratory method was chosen – Principal Components Analysis (PCA). PCA is a useful statistical technique to inspect if a number of variables are linearly related to a reduced number of unobservable dimensions. PCA uses common variance underlying the observed variables (i.e. the 69 food item preference scores) to identify a smaller number of 'latent factors', so-called 'principal components' (Everitt & Dunn 2001; Weiss 1970). This approach has previously been used to classify and interpret food preference patterns in samples of varying ages and nationalities (Newby & Tucker 2004). Decisions regarding the final factor structure of food preference data were also guided by currently accepted food categories.

4.3.6.2 Tests of the assumptions on PCA

The appropriateness of PCA as a data reduction technique is based on a number of assumptions being met (Field 2013):

[1] Sampling adequacy. This can be tested using the Kaiser-Mayer-Olkin (KMO) measure of sampling adequacy (Kaiser et al. 1970). The KMO index reflects the ratio of the sum of the partial correlations relative to the sum of the correlations of the observed variables. A KMO between 0.5 – 0.7 is considered 'mediocre', 0.8 – 0.9 considered 'great', and anything beyond as 'superb' for PCA (Hair et al. 1995).

[2] Sample size: A suitably large sample is necessary for PCA, with a minimum recommendation of 5-10 participants per variable up to a total sample size of 300. A sample size of >300 participants is generally classed as 'good', above which test parameters are stable (Comrey & Lee 1992).

[3] Sufficient collinearity between the measured variables: Variables need to show substantial correlations with each other, i.e. every variable shows inter-correlations with some but not all other variables. Calculation of inter-item correlations can be used to test whether this assumption is met (Field 2013). On the other hand, it is also important that multicollinearity (items correlating too highly: >r=0.9) and singularity (perfect

correlation between items) is avoided; this can be tested for by the determinant of the correlation matrix (a value of >0.00001 satisfying this assumption).

[4] Bartlett's Test of Sphericity is a statistical test that formally assesses the inter-correlations of the variables, testing the hypothesis that the correlation matrix of the dataset is an identity matrix – i.e. that there are sufficient correlations among the items in the dataset. A statistically significant result suggests that the null hypothesis (that there are no significant correlations among items) can be rejected, indicating that the variables are sufficiently correlated (overall) and PCA is appropriate (Bartlett 1950).

4.3.6.3 Extraction of components

There are two methods for identifying the number of components underlying the variables – selecting the components with the highest eigenvalue, or using a scree plot. An eigenvalue indicates the amount of variation in the dataset explained by each component. The process allows as many components as there are original variables, so only the components with a high enough eigenvalue should be selected. The higher the eigenvalue, the higher the percentage of variance explained by the variables loading onto the factor. Kaiser suggests identifying components with an eigenvalue > 1 (Kaiser 1960). Field has suggested that this is only accurate when <30 variables are being considered and communalities after extraction are >0.7, or in larger sample sizes (> 250) where communalities are <0.6 (Field 2013). A scree plot shows the number of extracted components along the x axis, with their associated eigenvalues along the y axis. As a general rule, the value just before the point of inflection, where the graph begins to steadily level out, it is said to give an indication of the optimum number of components (Cattell 1966). Extraction of components was based on Kaiser's criterion because the data met the criteria, and it offers a more objective method of choosing component number than a scree plot which can be very subjective.

4.3.6.4 Factor loading values

PCA generates factor loadings for each item. Factor loadings indicate the correlation between the component and the item; the square of the factor loading indicates the percentage of variance in the item explained by the factor. Items can load on to multiple components so each item has a factor loading for each component. There is no 'hard and fast rule' for deciding on the minimum factor loading value for an item to be included in a component. Many researchers suggest the minimum value should be 0.4 – these are items that the factor explains a minimum of 16% of the variance in (0.04^2) (Costello & Osborne 2005). However, for practical assessment of standardised factor loadings, other researchers have suggested that a factor loading cut-off of >0.30 is reliable in sample sizes >350 (Hair et al. 1995).

4.3.6.5 Rotation method

Following the initial calculation of item factor loadings, two different rotation techniques can be applied to even-out and improve interpretability of the obtained factor structure (Yaremko et al. 2013). Orthogonal rotation methods assume that the factors in the analysis are uncorrelated, whilst oblique rotation methods assume that factors are correlated.

Because the theoretical framework of food preferences suggests that food preference factors inter-correlate, an oblique rotation method (‘Direct Oblimin’) was chosen. With oblique rotation methods, two component matrices are obtained – the pattern matrix and the structure matrix. The pattern matrix displays the factor loadings together with the unique contribution of each item to each factor, while the structure matrix additionally takes into account the correlations between the variables and the factors. I will be presenting the structure matrix in the results section which is more complete than the pattern matrix as occasionally values are omitted from the latter if observed factors correlate (Graham et al. 2003).

4.3.6.6 Power for PCA

Power calculations for PCA are based on participant to questionnaire item ratios (Osborne & Costello 2004). There is no hard cut-off point for what ratios are considered acceptable for PCA, however recommendations range from a minimum participant to item ratio of at least 5:1 to 10:1 (Gorsuch 1983; Nunnally 1978). Accordingly, the TEDS dataset is very well-powered to produce reliable results in PCA (2865/69 = Ratio of 41.5:1)

4.3.6.7 Missing data

To maximize the data available for analysis, pairwise deletion was used to deal with missing observations. This maximizes the number of participants in the final analysis as all cases with any data are retained. This method is considered appropriate for large studies with limited missing data (Tabachnick et al. 2001). The only missing items in the dataset were the N/A responses (which were coded as missing) because participants had to complete every question on the online questionnaire.

4.3.6.8 Internal reliability analysis

Internal reliability of the components derived from the PCA was assessed using Cronbach’s α, the coefficient of reliability. In general, Cronbach’s α values of $\geq$ 0.7 indicate acceptable internal reliability for a psychometric scale; the closer the value to 1, the higher the internal reliability. Cronbach’s α values are calculated from pairwise correlations between all items included on a (sub)-scale, indicating how well items within a test approximate the same underlying latent component (Cronbach & Meehl 1955).

4.3.6.9 Food preference scale scores and drink item preference scores

Mean food preference scale scores were calculated by summing the single item scores within each food category, and dividing the sum by the total number of completed items in that category. Data for a minimum of 50% of items were required for calculation of food group scale scores. Drink preference scores were based on single item ratings due to the limited number of drink items included in the final questionnaire. Bivariate correlations were used to correlate mean group preferences for all six food preference categories, and for all seven beverage types. All analyses were undertaken using IBM SPSS Statistics for Windows, Version 22.0

4.4 Results

4.4.1 Sociodemographic characteristics of the main sample

The final eligible sample drawn from the TEDS study consisted of 2865 individuals. A more detailed description of the sociodemographic and anthropometric characteristics of the sample is available in **Chapter 3.**

4.4.2 Descriptive statistics for the food and drink items on the questionnaire

Food preference scores were positively skewed, indicating relatively high liking for most food items. The single most liked item was chocolate which had an average rating of 4.70 (SD=0.63) out of a maximum of 5. In contrast, the lowest preference score of 2.15 (SD=1.39) was found for liver. All items had been tried by >85% of the sample therefore all 69 food items were included in the PCA. Preference scores for the nine drink items showed that orange juice was the most liked drink with a mean preference score of 4.43 (SD=0.97). In contrast, beer was reportedly the least preferred drink (3.07 [SD=1.53]). Parametric and non-parametric paired samples t-tests indicated that all mean preference scores were significantly different from another (p<0.01). As shown in **Table 4.1**, bivariate correlations for all six food groups positively correlated with another (p<0.01). Correlations between drink preference scores were weaker, and the liking for coffee and fruit squash was significantly negatively correlated. For food preferences, vegetable and fruit liking had the strongest correlation (r=0.55), while liking for fizzy and diet fizzy drinks had the highest correlation for drink preferences (r=0.31).

Table 4.1 Pairwise correlation matrix showing the relationship between mean food category preference scores and mean drink preference scores

	Vegetables	Fruit	Meat/Fish	Dairy	Snacks	Starches	Fizzy drinks	Diet fizzy drinks	Fruit squash	Orange Juice	Milk	Coffee
Fruit	**0.55**** *(2863)*											
Meat/Fish	**0.39**** *(2863)*	0.29** *(2861)*										
Dairy	**0.42**** *(2865)*	0.28** *(2863)*	**0.42**** *(2865)*									
Snacks	0.05** *(2865)*	0.10** *(2863)*	0.18** *(2863)*	0.29** *(2865)*								
Starches	**0.37**** *(2865)*	**0.34*** *(2863)*	0.26* *(2863)*	**0.41**** *(2865)*	**0.31**** *(2865)*							
Fizzy drinks	-0.11** *(2841)*	-0.03 *(2839)*	0.07** *(2839)*	0.08** *(2841)*	0.36** *(2841)*	-0.01 *(2841)*						
Diet fizzy drinks	-0.02 *(2827)*	-0.01 *(2825)*	0.03 *(2825)*	0.11** *(2827)*	0.25** *(2827)*	0.04* *(2827)*	**0.31**** *(2821)*					
Fruit squash	0.00 *(2847)*	0.08** *(2845)*	0.05* *(2845)*	0.15** *(2847)*	**0.39**** *(2847)*	0.16** *(2847)*	0.27** *(2839)*	0.22** *(2825)*				
Orange Juice	0.12** *(2849)*	0.21** *(2847)*	0.13** *(2847)*	0.16** *(2849)*	0.22** *(2849)*	0.18** *(2849)*	0.21** *(2839)*	0.10** *(2824)*	0.27** *(2844)*			
Milk	0.04* *(2707)*	0.05** *(2705)*	0.16** *(2705)*	0.21** *(2707)*	0.17** *(2707)*	0.21** *(2707)*	0.12** *(2699)*	0.04* *(2684)*	0.15** *(2703)*	0.14** *(2703)*		
Coffee	0.12** *(1905)*	0.08** *(1905)*	0.08** *(1904)*	0.09** *(1905)*	0.01 *(1905)*	0.05* *(1905)*	0.00 *(1902)*	0.04 *(1891)*	-0.06* *(1902)*	0.02 *(1904)*	0.02 *(1837)*	
Tea	0.10** *(2415)*	0.09** *(2414)*	0.07** *(2413)*	0.09** *(2415)*	0.05* *(2415)*	0.10** *(2415)*	0.00 *(2407)*	-0.02 *(2395)*	-0.02 *(2410)*	0.02 *(2412)*	0.11** *(2330)*	0.18** *(1796)*

**Correlation is significant at the 0.01 level (2-tailed)
Pearson correlation coefficients >0.3 (**bolded**) suggest a medium effect size
Sample size given in brackets

4.4.3 Data screening for Principal Components Analysis

PCA was only used for the reduction of the food preference data. This was because drink preferences had been assessed using fewer items (n=9) that represented broader categories, and there was no need to further reduce these components. Factorability of the food preference data was adequate; the determinant of the correlation matrix was >0.00001 indicating that no extreme collinearity was present in the dataset. The overall KMO was 0.921, indicating 'superb' sampling adequacy (Kaiser 1974). Factorability of the data was further supported by a significant Bartlett's Test of Sphericity (χ^2 (1953) = 38499.201, $p<0.0001$). These results indicated that the basic assumptions necessary for PCA were met.

4.4.3.1 Components of the food preference questionnaire

The initial PCA was run on the entire food preference dataset. There were six components that had eigenvalues of >1: Component I (11.898), Component II (5.648), Component III (3.190), Component IV (2.528), Component V (2.131) and Component VI (1.891). The six components together accounted for 39.547% of the total observed variance in the 69 food preference scores.

Table 4.2 shows the factor loading scores for the 69 items for each of the six components, from the Structure Matrix. Marmite was excluded from the food groups because it is a condiment and did not fit coherently with the other foods.

Six items loaded onto two components (i.e. they had factor loadings >0.4 on two components): liver, smoked salmon and oily fish all loaded onto Components III (mainly meat and fish) and IV (mainly dairy foods); sugared cereal and bread both loaded onto Components II (mainly snack foods) and VI (mainly starches); apricots loaded onto Components IV (mainly dairy foods) and V (mainly fruits). Liver, smoked salmon and oily fish were all included in Component III, because the other foods in this component consisted mainly of meat or fish. Sugared cereal was included in Component II (mainly snack foods) not Component VI (mainly starch foods) as sugared cereals are predominantly liked for their sweet taste rather than as a savoury starch food. On the other hand, bread was included in Component VI (mainly starch foods) not Component II (mainly energy dense snack foods) as it did not fit logically with any of the items in the snack category. Apricots were included in Component V (fruits) rather than Component IV (dairy) because apricot is a fruit, and tends to be eaten in the same way as other fruits in the group.

There were four items (rice, custard, mayonnaise, yoghurt) with factors loadings over >0.3 onto multiple components, and these food items were included in the Component with the highest loading. For a few items, inclusion was based on matching food types. Rice was included in Component VI, which consisted of five other starchy foods as it did not fit with the items in the vegetable category. Yoghurt was included in Component V, which consisted of dairy foods rather than Component V (fruit) or Component VI (starch foods).

Finally, avocado and baked beans were repositioned for theoretical reasons. Avocado loaded onto Component VI (dairy), but ended up being included in Component I (mainly vegetables). Although avocado is technically classed as a fruit, it is not eaten in the same context or preparation style as most other fruits and it does not taste sweet (a characteristic of most fruit); it tends to be consumed alongside vegetables. Baked beans loaded onto Component VI (starch foods) but were included in Component I which consisted mainly of vegetables, as baked beans are considered a vegetable in daily intake guidelines.

For theoretical reasons, a small number of non-main meal composite food items were omitted from the final factor structure in order to increase the logical structure of the components: apple pie was excluded from 'fruits' and vegetable soup was excluded from 'vegetables' as they are both composite foods, coriander was excluded from 'vegetables' as it is a herb and not a food per se, and peanut butter and nuts were excluded from 'dairy' as they did not conceptually fit with the other foods in the 'dairy' category. In total 63 food items were retained in the final food preference questionnaire.

'Traditional' food group labels were used to name each of the six factors: vegetables, snacks, meat/fish, dairy, fruit, and starches. Allocated category labels were largely relevant to most items loading on to each factor, respectively. Regrettably, fit of the category label for few single items was odd e.g. eggs loading on to 'dairy', hummus loading on to 'meat/fish', etc. Despite this slightly imperfect fit, the labels were useful to define a clear structure, allow easier interpretation of food preference groups, and facilitate quantitative comparisons to previous research studies that have used similar groupings of food preferences.

Table 4.2 Factor structure of food item preference scores

Structure Matrix						
	Factor loading values[1]					
	I. Vegetables	II. Snacks	III. Meat/Fish	IV. Dairy	V. Fruit	VI. Starches
Green beans	**.726**	.009	-.096	.247	-.345	.101
Broccoli	**.686**	.000	-.086	.143	-.271	.128
Peas	**.635**	.067	-.096	.101	-.183	.144
Vegetable Soup	**.613**	.040	-.014	.358	-.327	.184
Salad	**.602**	.047	-.044	.212	-.422	.105
Carrots	**.597**	.080	-.076	.071	-.273	.119
Spinach	**.580**	-.106	-.010	.520	-.344	.081
Sweet Corn	**.574**	.093	-.067	.148	-.259	.139
Red pepper	**.565**	.029	-.020	.265	-.387	.049
Brussel sprouts	**.561**	-.049	-.082	.337	-.191	.047
Cucumber	**.555**	.097	-.065	.153	-.464	.014
Parsnips	**.546**	-.008	-.113	.368	-.254	.058
Beetroot	**.468**	-.076	-.027	.456	-.311	-.014
Celery	**.456**	-.041	-.024	.333	-.332	.016
Raw tomato	**.451**	.020	-.046	.388	-.449	-.035
Mushrooms	**.446**	-.041	-.097	.412	-.239	-.027
Coriander	**.436**	-.048	-.039	.416	-.288	.106
Rice	.337	.153	-.119	.026	-.145	**.309**
Choc. Biscuits	.060	**.691**	-.152	.007	-.036	.178
Chocolate	.095	**.653**	-.096	.021	-.048	.071
Cake	.081	**.633**	-.181	.073	-.081	.107
Crisps	-.009	**.623**	-.143	-.057	.032	.164
Ice cream	.046	**.603**	-.150	.072	-.096	.105
Chips	.019	**.581**	-.182	-.065	.056	.301
Plain biscuits	.110	**.560**	-.165	.021	-.067	.342
Butter	.066	**.491**	-.222	.218	.110	.343
Gummy sweets	-.054	**.485**	-.294	-.078	-.059	.149
Cream	.117	**.460**	-.245	.369	-.059	.298
Butter-like spread	.064	**.423**	-.106	.155	.111	.374
Custard	.195	.306	-.162	**.300**	-.180	.285
Beef	.091	.183	**-.831**	.057	-.021	.085
Beef burgers	.034	.222	**-.805**	.035	.027	.100
Bacon	-.032	.232	**-.775**	-.017	.006	.118
Ham	.031	.226	**-.773**	.003	-.013	.165
Sausages	.045	.279	**-.767**	-.028	.021	.166
Chicken	.009	.219	**-.766**	-.105	-.017	.094
Lamb	.093	.113	**-.714**	.153	-.082	.042

Structure Matrix

	Factor loading values[1]					
	I. Vegetables	II. Snacks	III. Meat/Fish	IV. Dairy	V. Fruit	VI. Starches
White fish	.306	.067	**-.516**	.316	-.138	.019
Liver	.154	.054	**-.481**	**.416**	-.085	.036
Tinned tuna	.292	.107	**-.409**	.360	-.179	.040
Soft cheese	.221	.132	-.124	**.652**	-.127	.161
Hummus	.344	-.047	.028	**.607**	-.319	.076
Cottage cheese	.198	.044	-.099	**.598**	-.123	.169
Avocado	.341	-.108	.005	**.589**	-.377	.017
Smoked salmon	.299	-.006	**-.433**	**.549**	-.222	-.067
Oily fish	.278	-.026	**-.416**	**.547**	-.219	-.067
Peanut butter	.200	.035	-.019	**.514**	-.250	.142
Nuts	.296	-.025	.002	**.468**	-.349	.202
Mayonnaise	.186	.303	-.220	**.390**	-.058	.171
Hard cheese	.161	.294	-.157	**.376**	-.048	.259
Marmite	.170	-.025	-.032	.349	-.162	.030
Eggs	.219	.150	-.280	**.349**	-.173	.248
Peaches	.348	.004	-.060	.350	**-.772**	.098
Grapes	.319	.069	-.049	.045	**-.711**	.171
Oranges	.321	.047	-.080	.128	**-.692**	.154
Strawberries	.287	.098	-.077	.170	**-.687**	.051
Apricots	.326	-.014	-.064	**.418**	**-.687**	.114
Melon	.336	.036	-.055	.222	**-.595**	.083
Apples	.317	.115	-.082	-.028	**-.559**	.223
Apple Pie	.266	.276	-.171	.341	**-.430**	.142
Yogurt	.252	.003	-.049	**.326**	-.349	.331
Wheat cereal	.172	.129	-.087	.150	-.157	**.701**
Rice/corn cereal	.121	.292	-.137	.030	-.083	**.683**
Bran cereal	.233	-.034	-.021	.295	-.306	**.558**
Sugared cereal	-.060	**.436**	-.180	.006	.039	**.534**
Bread	.144	**.426**	-.138	-.040	-.048	**.476**
Porridge	.301	-.045	-.058	.384	-.330	**.462**
Potatoes	.324	.331	-.175	-.027	-.001	**.406**
Baked beans	**.295**	.204	-.157	.186	-.100	.314

4.4.3.2 Reliability analysis

Cronbach's α was used to assess the internal reliability of the final six food categories:.. Internal consistency was good for each of 'vegetables' (α=0.86; 17 items), 'fruits' (α=0.82; 7 items), 'meat/fish' (α=0.79; 13 items), 'dairy' (α=0.75; 10 items), and 'snacks' (α=0.73; 9 items); but α was just below the threshold of 0.7 for starch foods (α=0.69; 7 items). The Cronbach's α coefficients for each food group, and the items included in each food group are shown in **Table 4.3.**

Table 4.3 The 68 food items grouped across initial six food preference components with Cronbach α values for overall and item specific scores (excluding Marmite)

Vegetables	α[1]	Fruit	α[1]	Meat/Fish	α[1]	Dairy	α[1]	Snacks	α[1]	Starches	α[1]
Spinach	0.869	Oranges	0.796	Beef	0.787	Eggs	0.750	Chips	0.752	Bread	0.673
Carrots	0.875	Grapes	0.800	Beef burgers	0.791	Soft cheese	0.730	Plain biscuits	0.751	Bran cereal	0.634
Green beans	0.870	Apples	0.818	Lamb	0.786	Hard cheese	0.743	Choc. biscuits	0.737	Porridge	0.659
Cucumber	0.873	Melon	0.818	Chicken	0.802	Butter	0.747	Cake	0.752	Rice	0.681
Celery	0.875	Peaches	0.771	Bacon	0.795	Cream	0.735	Ice cream	0.753	Wheat cereal	0.616
Mushrooms	0.875	Apricots	0.791	Ham	0.792	Yoghurt	0.755	Chocolate	0.750	Rice/corn cereal	0.683
Brussels sprouts	0.873	Strawberries	0.800	Sausages	0.798	Cottage cheese	0.737	Crisps	0.749	Potatoes	0.639
Parsnips	0.872	Apple pie[2]	0.879	White fish	0.786	Butter-like spread	0.750	Gummy sweets	0.763		
Peas	0.874			Tinned tuna	0.799	Mayonnaise	0.745	Sugared cereal	0.766		
Sweetcorn	0.875			Oily fish	0.782	Custard	0.751				
Broccoli	0.872			Smoked salmon	0.782	Peanut butter[2]	0.753				
Salad	0.873			Hummus	0.807	Nuts[2]	0.749				
Red pepper	0.873			Liver	0.790						
Raw tomato	0.873										
Avocado	0.874										
Baked beans	0.881										
Beetroot	0.872										
Veg soup[2]	0.871										
Coriander[2]	0.874										
17 items		**7 items**		**13 items**		**10 items**		**9 items**		**7 items**	
Final Cronbach's α											
0.86		0.82		0.79		0.75		0.73		0.69	
(n=2865)		(n=2862)		(n=2855)		(n=2865)		(n=2860)		(n=2864)	

[1] Cronbach α if item deleted from scale
[2] Denotes items that were removed post-PC

4.4.4 Food group preference scale scores

Out of the original 69 food preference items, 63 items were retained which represented six common food groups: vegetables, fruits, meat/fish, dairy foods, snacks and starch foods. 59 of the 63 items had been tasted by over 90% of the study subjects, the exceptions being hummus, avocado, cottage cheese and liver. Food group scores indicated that snack foods were the most popular with a mean of 4.39 (SD=0.44), and vegetables the least liked category with a mean score of 3.59 (SD=0.78). Results are summarized in **Table 4.4.**

Table 4.4 Food preference scores, factor loadings and Cronbach's for the 63 items retained in the six components

Food Items	**Eligible Adolescents** (% who have tried the food)	**Mean preference score**[1] (SD)	**Factor Loadings**	**Cronbach's α**
Vegetables (17 items)	2865 (100)	3.59 (0.78)		**0.864**
Carrots	2851 (99.5)	4.30 (1.01)	0.60	
Sweetcorn	2842 (99.2)	4.22 (1.19)	0.55	
Salad	2848 (99.4)	4.16 (1.07)	0.60	
Peas	2848 (99.4)	4.07 (1.26)	0.64	
Broccoli	2831 (98.8)	4.05 (1.24)	0.69	
Cucumber	2843 (99.2)	4.05 (1.29)	0.56	
Red pepper	2825 (98.4)	4.04 (1.28)	0.57	
Baked beans	2844 (99.3)	3.95 (1.27)	0.30	
Green beans	2816 (98.2)	3.90 (1.23)	0.73	
Spinach	2686 (93.8)	3.44 (1.43)	0.58	
Parsnips	2774 (96.8)	3.40 (1.54)	0.55	
Raw tomato	2840 (99.1)	3.30 (1.66)	0.45	
Mushrooms	2826 (98.7)	3.26 (1.67)	0.45	
Avocado	2455 (85.7)	2.94 (1.50)	0.34	
Celery	2770 (96.7)	2.91 (1.52)	0.46	
Beetroot	2681 (93.6)	2.79 (1.58)	0.47	
Brussels sprouts	2801 (97.8)	2.77 (1.58)	0.56	
Fruit (7 items)	2863 (99.9)	4.19 (0.80)		***0.824***
Grapes	2855 (99.7)	4.63 (0.82)	-0.71	
Apples	2860 (99.8)	4.55 (0.80)	-0.56	
Strawberries	2853 (95.5)	4.55 (0.98)	-0.69	
Oranges	2859 (99.8)	4.31 (1.01)	-0.69	
Melon	2839 (99.0)	4.06 (1.29)	-0.6	
Peaches	2795 (97.6)	4.00 (1.56)	-0.77	
Apricots	2736 (95.5)	3.56 (1.36)	-0.69	
Meat/Fish (13 items)	2855 (99.9)	3.97 (0.77)		***0.788***
Chicken	2767 (96.6)	4.75 (0.65)	-0.77	
Bacon	2724 (95.0)	4.44 (1.05)	-0.78	
Sausages	2752 (96.0)	4.33 (1.03)	-0.77	
Beef burgers	2739 (95.6)	4.32 (1.07)	-0.81	
Beef	2749 (96.0)	4.29 (1.06)	-0.83	
Ham	2724 (95.0)	4.14 (1.14)	-0.77	
White fish	2776 (96.6)	3.97 (1.31)	-0.52	
Lamb	2721 (95.0)	3.90 (1.35)	-0.78	
Tinned tuna	2755 (96.2)	3.69 (1.56)	-0.41	
Smoked salmon	2678 (93.5)	3.37 (1.62)	-0.43	

Hummus	2497 (87.2)	3.36 (1.56)	0.03	
Oily fish	2626 (91.7)	2.82 (1.51)	*0.42*	
Liver	2453 (85.6)	2.15 (1.39)	-0.48	
Dairy (10 items)	2865 (100)	3.62 (0.73)		***0.749***
Hard cheese	2847 (99.4)	4.23 (1.13)	0.38	
Eggs	2836 (99.0)	4.14 (1.25)	0.35	
Butter	2838 (99.1)	3.97 (1.10)	0.22	
Custard	2845 (99.3)	3.94 (1.35)	0.30	
Butter-like spread	2832 (98.8)	3.80 (1.13)	0.46	
Cream	2832 (98.8)	3.76 (1.24)	0.37	
Yoghurt	2805 (97.9)	3.67 (1.23)	0.33	
Mayonnaise	2828 (98.8)	3.59 (1.43)	0.56	
Soft cheese	2766 (96.6)	3.39 (1.47)	0.65	
Cottage cheese	2566 (89.6)	2.40 (1.39)	0.60	
Snacks (9 items)	2865 (100)	4.39 (0.55)		***0.731***
Chocolate	2855 (99.7)	4.70 (0.63)	0.65	
Chocolate biscuits	2854 (99.6)	4.57 (0.76)	0.69	
Chips	2861 (99.9)	4.54 (0.76)	0.58	
Ice cream	2851 (99.5)	4.53 (0.81)	0.60	
Cake	2854 (99.6)	4.52 (0.83)	0.63	
Crisps	2855 (99.7)	4.46 (0.83)	0.62	
Plain biscuits	2854 (99.6)	4.22 (0.91)	0.56	
Gummy sweets	2833 (98.9)	4.14 (1.17)	0.49	
Sugared cereal	2815 (98.3)	3.93 (1.13)	0.44	
Starches (7 items)	2865 (100)	3.88 (0.70)		***0.690***
Bread	2859 (99.8)	4.50 (0.74)	0.48	
Potato	2860 (99.8)	4.30 (0.94)	0.41	
Rice/Corn cereal	2854 (99.6)	4.04 (1.00)	0.68	
Wheat cereal	2836 (99.0)	3.98 (1.09)	0.70	
Rice	2851 (99.5)	3.96 (1.04)	0.31	
Porridge	2811 (98.1)	3.53 (1.37)	0.46	
Bran cereal	2790 (97.4)	3.46 (1.24)	0.56	

4.4.5 Drink item preference scores

Table 4.5 shows the percentages of participants who consumed each drink type and the mean preference score for each drink item.

4.4.6 Drink consumption

In comparison to most of the foods being tasted by most of the participants, there was greater variability in the percentages of the participants who consumed the nine beverage types measured in the questionnaire. Coffee was the least consumed drink (66.5%), followed by wine (69.6%) and tea (84.3%). The remaining beverages were all consumed by over 90% of the participants.

Table 4.5 Percentage of study participants that consume the nine beverage types and mean drink preference scores

Drink Items	**Consumers** (%)	**Mean preference score**[1] (SD)
SSBs	2841 (99.2)	3.73 (1.37)
NNSBs	2827 (98.7)	3.64 (1.34)
Orange juice	2849 (99.5)	4.43 (0.97)
Fruit squash	2847 (99.4)	4.23 (1.02)
Milk	2707 (94.5)	4.23 (0.95)
Coffee	1905 (66.5)	3.85 (1.29)
Tea	2415 (84.3)	4.31 (1.08)
Wine	2001 (69.8)	3.58 (1.25)
Beer	2578 (90.0)	3.07 (1.57)

[1] Preference scores were calculated for participants that reported consuming the beverage
Abbreviations: SSB=Sugar-sweetened beverage; NNSBs=Non-nutritive sweetened beverages

4.4.7 Test-retest results

4.4.7.1 Socio-demographic characteristics of test-retest sample

Of the 205 siblings invited to trial the food preference questionnaire, n=124 (60.5%) siblings completed the first questionnaire; 94/124 (75.8%) participants completed both waves of data collection. The mean age of test-retest sample was 17.52 (SD=0.59) indicating that the sample was appropriate to test suitability and reliability of the questionnaire in an adolescent sample. The sibling sample was also representative in terms of sex (n=86; 69.4% female).

4.4.8 Test-retest pilot feedback

Qualitative responses collected during the test-retest study indicated that the questions and instructions on the food and drink preference questionnaire were well understood. No comprehension difficulties or complaints regarding the length of the questionnaire were reported by the 124 test-retest pilot study participants that had completed at least one round of data collection.

Table 4.6 Food and drink preference questionnaire item test-retest reliability coefficients

Food and drink items	**Test-retest ICC**	**95% CI**	
Vegetables (n=17)	0.950	0.926	0.967
Spinach	0.872	0.812	0.913
Carrots	0.801	0.713	0.863
Green beans	0.856	0.790	0.902
Cucumber	0.875	0.818	0.916
Celery	0.844	0.773	0.894
Mushrooms	0.928	0.893	0.952
Parsnips	0.889	0.837	0.925
Peas	0.911	0.868	0.940
Sweetcorn	0.864	0.801	0.908
Broccoli	0.880	0.824	0.919
Salad leaves (e.g., lettuce)	0.859	0.794	0.904
Red peppers	0.893	0.843	0.928
Raw tomatoes	0.953	0.931	0.969

Beetroot	0.879	0.823	0.918
Brussels sprouts	0.941	0.911	0.960
Baked beans	0.889	0.837	0.925
Avocado	0.834	0.760	0.887
Fruit (n=7)	0.843	0.772	0.894
Oranges	0.849	0.780	0.898
Grapes	0.851	0.783	0.899
Apples	0.799	0.711	0.862
Melon	0.854	0.787	0.901
Peaches	0.801	0.714	0.864
Apricots	0.743	0.636	0.822
Strawberries	0.821	0.741	0.878
Meat/Fish (n=13)	0.949	0.924	0.966
Beef	0.868	0.807	0.911
Beef burgers	0.869	0.808	0.911
Lamb	0.869	0.808	0.911
Chicken	0.829	0.752	0.883
Bacon	0.878	0.821	0.918
Ham	0.912	0.870	0.941
Liver	0.730	0.618	0.813
Sausages	0.934	0.902	0.956
White fish (e.g. cod, haddock)	0.852	0.784	0.899
Oily fish (e.g. mackerel, kippers)	0.815	0.732	0.873
Smoked salmon	0.848	0.779	0.897
Tinned Tuna	0.938	0.908	0.959
Hummus	0.913	0.872	0.942
Dairy (n=10)	0.902	0.856	0.934
Eggs (boiled, scrambled or fried)	0.920	0.882	0.946
Soft cheese (e.g. Camembert, Brie)	0.852	0.784	0.900
Hard cheese (e.g. Cheddar)	0.883	0.828	0.921
Cottage Cheese	0.684	0.558	0.779
Plain, low-fat yoghurt	0.704	0.584	0.794
Custard	0.930	0.896	0.953
Butter	0.701	0.580	0.791
Butter-like spreads (e.g. Sunflower spread, Flora)	0.610	0.463	0.723
Cream	0.787	0.695	0.854
Mayonnaise	0.925	0.888	0.950
Snacks (n=9)	0.842	0.770	0.892
Plain biscuits (e.g. digestives)	0.711	0.593	0.799
Chocolate biscuits	0.724	0.611	0.809
Cake	0.727	0.614	0.811
Ice cream	0.613	0.468	0.726
Sugared cereal (e.g. Frosties, Sugar Puffs)	0.790	0.699	0.856
Chocolate	0.695	0.572	0.787
Chips	0.773	0.675	0.844
Crisps	0.752	0.648	0.829
Chewy gummy sweets (e.g. Haribo-style sweets)	0.846	0.775	0.895
Starches (n=7)	0.818	0.737	0.876
Bread or Bread rolls	0.689	0.565	0.783
Rice or corn cereal (e.g. Corn Flakes, Rice Krispies)	0.723	0.609	0.808
Bran cereal (e.g., All Bran, Bran Flakes)	0.708	0.589	0.797
Porridge	0.793	0.703	0.858
Plain boiled rice	0.807	0.722	0.868
Wheat cereal (e.g., Weetabix, Shredded Wheat)	0.726	0.613	0.810
Potatoes (boiled or mashed)	0.785	0.692	0.853
Drink types (n=9)			

Milk	0.854	0.787	0.902
Coffee	0.765	0.621	0.859
Tea	0.691	0.554	0.792
Non-diet fizzy drinks (e.g. Coca Cola, Pepsi)	0.857	0.792	0.903
Diet fizzy drinks (e.g. Diet Coke, Pepsi Max)	0.927	0.892	0.951
Orange juice	0.717	0.602	0.803
Fruit squash (e.g., Ribena, orange squash)	0.888	0.835	0.924
Wine	0.880	0.794	0.932
Beer	0.938	0.908	0.959
Uncategorized (n=13)			
Marmite	0.914	0.872	0.942
Nuts (e.g., almonds, brazil nuts)	0.838	0.765	0.890
Apple pie	0.909	0.866	0.939
Peanut butter	0.913	0.871	0.941
Vegetable soup	0.864	0.802	0.908
Coriander (aka cilantro)	0.766	0.667	0.839
Sweet tooth?	0.818	0.737	0.875
Spicy (hot) food?	0.925	0.889	0.950
Frequency of salt addition	0.872	0.813	0.913
Milk in tea? [Black/White]	0.917	0.873	0.946
Sweetened tea? (Unsweetened/ Sweetened)	0.920	0.878	0.949
Milk in coffee? [Black/ White]	0.729	0.569	0.836
Sweetened coffee? [Unsweetened/ Sweetened]	0.959	0.930	0.977

4.4.8.1 Test-retest reliability

Mean preference test-retest scores indicated that responses were reliable over a 2-week period. Test-retest coefficients for individual food and drink preference scores ranged from 0.61 for ‘butter-like spread’ to 0.96 for wine (**Table 4.6**). Based on the food categories obtained from the PCA, high stability of mean food group preference scores was also demonstrated as indicated by high and significant test-retest coefficients as follows: ‘vegetables’ (ICC=0.95), ‘fruit’ (ICC=0.84), ‘meat/fish’ (ICC=0.95), ‘dairy’ (ICC=0.90), ‘snacks’ (ICC=0.84), and ‘starches’ (ICC=0.82). Mean test-retest coefficients for beverage preferences were moderate to high: tea (ICC=0.69), orange juice (ICC=0.72), coffee (ICC=0.77), milk (ICC=0.85), non-diet fizzy drinks (ICC=0.86), fruit squash (ICC=0.89), and diet fizzy drinks (ICC=0.93).

4.5 Discussion

This study describes the development of a comprehensive questionnaire to measure reliably the food and drink preferences of older British adolescents. The question content and online data collection platform were appropriate and well-received in self-report format.

4.5.1 Summary of findings

These findings show that food item preference scores are inter-related in older adolescents. Based on PCA, preferences for 63 foods measured using a self-reported preference checklist, could be grouped into six internally reliable and logical categories: vegetables, fruits, meat/fish, dairy, snacks and starches. This study confirms, as has previously been shown in children, the underlying structure of adolescents' food preferences largely reflects traditional food categories and does not necessarily directly correspond to specific tastes (Wardle, Sanderson, et al. 2001). This is demonstrated by preferences for food items with different taste profiles, such as sweet (e.g. chocolate) or salty (e.g. crisps), loading on to common components (i.e. snacks).

Findings from the small test-retest study indicated the questionnaire was able to measure these characteristics consistently over time. This suggests that a single administration of the preference questionnaire can reliably measure preferences for common foods and drinks in this population.

Lower preference for micronutrient dense foods such as vegetables has detrimental implications for health, if this leads to lower intake. The findings the vegetables were the least liked food in this study relates to the results seen in two previous twin studies looking at similar food groups in children (Fildes, van Jaarsveld, Llewellyn, et al. 2014; Breen et al. 2006). Potential negative health outcomes are strengthened further if these healthier foods are displaced in the diet with well-liked processed 'junk' foods high in fat, sugar and salt. Snack foods were the most preferred food group among adolescents in this study. This is concerning given that the items in the snack category are exclusively energy-dense, nutrient poor foods (e.g. biscuits, chocolate, crisps). Older adolescents are known to adopt food habits not consistent with the recommended dietary guidelines so a high preference for energy dense foods was expected (Kimmons et al. 2009; Guenther et al. 2006). This finding further reiterates the need for interventions to shift adolescents' unhealthy food preferences towards more nutritious choices.

Fruit was the second most popular food group, closely behind snack foods. This may be explained by the fact that fruits tend to be sweet. People innately prefer sweet tastes which may explain why preference scores for this category was so high (Beauchamp 2016). Importantly however, in comparison to sweet snack food, fruits are low in energy, and provide fibre, vitamins and minerals - essential nutrients that make up a healthy diet so a higher preference for these foods reflects a healthier preference. On the other hand, vegetables were the least preferred food group in this sample. This pattern is reflected in dietary intake data from adolescent populations (Kimmons et al. 2009; Guenther et al. 2006; Minaker & Hammond 2016). Even though in the US, the overall proportion of adolescents meeting the recommended amount of two servings of fruit and three vegetables is very low (0.9%), the proportion of 12-18 year olds meeting the fruit recommendations is considerably higher (6.2%) compared to vegetable intake (2.2%) (Kimmons et al. 2009), potentially reflecting higher liking for fruits compared to vegetables. Current UK dietary guidelines recommend that individuals consume at least 5 portions of fruit and vegetables a day but do not make specific recommendations for fruit or vegetables separately (Public Health England 2016). The significantly higher mean preference observed for fruits compared to vegetables in this sample suggests it may be harder to encourage adolescents to increase their consumption of vegetables, than for more well-liked fruits (see **Study 2**). Given vegetables are less preferred, more intensive efforts may be needed to increase their intake (Yeh et al. 2008).

The results also demonstrate the wide variety of food and drink types that adolescents in the UK experience by the time they enter adulthood; 59 out of 63 food items were known to over 90% of participants (the only exceptions being avocado, hummus, cottage cheese and liver). Coffee was by far the least frequently consumed beverage with only 66.5% of participants reporting drinking coffee. This might reflect the younger age of the cohort, as it has previously been suggested individuals gradually develop an affinity for more bitter foods and flavours (e.g. coffee) as they enter adulthood (Cowart et al. 1994)

Food preference assessment tools have largely focused on measuring the dietary preferences of young children and are usually completed by a parent on behalf of their child (Breen et al. 2006; Fildes, van Jaarsveld, Llewellyn, et al. 2014). Previous food preference questionnaires for adults were mainly developed with the aim of assessing a narrow range of taste preferences, or liking for specific food or drink items implicated in particular health problems. For example, a food preference questionnaire developed by Geiselman et al (1998) was created with the purpose of identifying men with a high liking for fattiness in food because of the implications of high dietary fat consumption for cardiovascular health and other diseases (Geiselman et al. 1998). Similarly, the food items on two food questionnaires used by Duffy and colleagues had been chosen to measure liking for foods high in fat (e.g. mayonnaise), fatty foods with sweet tastes (e.g. cookies), salty foods (e.g. bacon) and bitter foods or drinks (e.g. broccoli, coffee) (Duffy et al. 2007; Duffy et al. 2009). Some of these questionnaires have been gender-specific, limiting their utility for large scale population research (Geiselman et al. 1998; Deglaire et al. 2014).

It is also important to consider that previous dietary preference questionnaires have largely neglected liking for different drink types. Measuring drink preferences is especially important in older adolescents given that 18% of the daily caloric intake of

UK adolescents is now attributable to drinks (Ng et al. 2012). Because many of these previous tools have strongly focused on the assessment of liking for a narrow range of dietary items, the questionnaire presented in this study provides a necessary comprehensive solution to measure and evaluate food and drink preferences patterns in (young) adults.

4.5.2 Strengths and limitations

Food preference scores were obtained from a large sample with a narrow age range. This is important as food and drink preferences vary widely over the lifespan (Koehler & Leonhaeuser 2008). However, TEDS is a predominantly white, high social class British cohort and twins themselves are usually slightly leaner than the general population (Estourgie-van Burk et al. 2010). Food and drink preferences in this sample may not therefore be representative of the wider population.

Although the food preference categories were derived using a robust statistical technique, there were number of food items that did not map well on to the six food components. This required post-hoc re-assignment of some items to alternative components to improve interpretability of the questionnaire (e.g. apricots loaded on to the ‘dairy’ component but was re-assigned to the ‘fruit’ component, exclusion of nuts and peanut butter from ‘dairy’ etc.). Therefore the final six food preference categories do not capture the entire variance of the original items (Weiss 1970). In addition, it was not possible to apply a similar factor analytic approach to investigate the patterns of drink preferences due to the limited number of beverages included in the questionnaire.

Because the data from PCA is time and sample-specific, replication of the factor structure in other populations of a similar age will be necessary to confirm the construct validity of the questionnaire. However it should be noted that the six factor solution, representing six distinct and logical food preference categories, obtained in this study directly reflects the structure of a similar food preference questionnaire for young children, despite some differences in the food items included (Fildes, van Jaarsveld, Llewellyn, et al. 2014). To further assess how strongly self-reported dietary preferences are correlated with dietary intake, comparison of the food preference questionnaire with dietary food recall data would strengthen convergent validity of the questionnaire. A perhaps more novel and accurate approach to assess convergent validity of the questionnaire and actual dietary intake could also integrate the use of innovative approaches such as the Remote Food Photography Method (Martin et al. 2012). This method can record dietary intake in the natural environment, instructing individuals to use a camera-enabled phone to capture photos of any food or drink item consumed over a specified timeframe. Images are collected by researchers and with the help of a validated computer program, energy and nutrient intake can be estimated fairly accurately.

The food preference categories had moderate to high internal reliability. However, Cronbach alpha values are highly sensitive to the number of items in the scale. Starches, the food preference scale with the smallest number of items (n=7) had a Cronbach α of 0.69, just below the threshold value of 0.7 (the value considered to index a scale of moderate internal consistency). This slightly lower internal consistency may partly reflect the fewer number of food items in this category.

Preference scores were based on self-report, which could be liable to underreporting of true liking for foods typically considered as “unhealthy”. This type of underreporting has sometimes been shown to be more prevalent in individuals with a higher BMI (Livingstone et al. 1992; Price et al. 1997; Bratteby et al. 1998). However, most participants in this study had a healthy BMI, and the questionnaire data were collected anonymously. This may therefore have helped to mitigate against social desirability bias in this sample.

Food preferences are also susceptible to short-term variation, influenced by recent consumption or exposure to certain tastes or diets in the period preceding measurement (Martin et al. 2011; Griffioen-Roose, Hogenkamp, et al. 2012; Griffioen-Roose, Mars, et al. 2012). However, the test-retest reliability analyses indicate this questionnaire was able to tap into general food preferences, without being strongly influenced by short-term variability in consumption patterns.

Another limitation of the study is the use of 5-point Likert scales, which may provide limited sensitivity in detecting variability of food and drink preferences. As a result, it is difficult to discriminate between individuals, especially for food items which are strongly skewed. If a food item is either strongly liked or disliked by the majority of people, the limited response options can result in ceiling effects, which means that information on variance at the extreme ends of preference scores is not detected. Ceiling effects, seen for the most popular food items (e.g. chocolate; 86.8% rated as ‘like a lot’) may compromise conclusions derived from the food categories on which such foods loaded.

A key strength of the present study was the high number of food items (n=69) contained within the questionnaire, representing foods and drinks commonly consumed by this demographic. The majority of food items were familiar to most study participants, with 59/63 foods known to >90% of the study subjects. This emphasizes the relevance of the food categories derived for measuring differences in adolescents’ food preferences.

4.6 Conclusion

In summary, this study describes the development of a comprehensive food and drink preference questionnaire, providing a relevant and reliable tool for measuring likes and dislikes for a range of food and beverage categories in older adolescents. These preference scores will enable me to investigate the relative importance of genetic and environmental influences on variation in adolescents' food and drink preferences. Understanding the aetiology of these traits will give better insights into the factors that shape them, informing strategies for promoting healthy preferences. The food and drink preference questionnaire also provides a measurement tool that is cheap, quick and easy to disseminate which may encourage future studies to further characterize causes or consequences of older adolescents' dietary preferences.

The questionnaire characterised a general factor structure of what foods and drinks adolescents prefer. **Study 2** aims to quantify the aetiology of these dietary preferences. With greater liking of energy-dense food and drinks implicated as risk factors for overweight and a range of negative health outcomes, **Study 3** investigates the relationship between food and drink preferences with adiposity in older adolescents. A questionnaire such as the one developed in this study can also be used to measure changes in dietary preferences after participating in a healthy lifestyle intervention. A shortened version of the food and drink preference questionnaire was used in **Study 4** to track food and drink preferences during a sugar reduction study in tea.

The food and drink preference questionnaire developed in this study forms the basis of the remaining studies in this thesis.

Chapter 5.

Study 2 - Genetic and environmental influences on food and non-alcoholic drink preferences in adolescence[2]

5.1 Introduction

As established in Chapter 1, twin studies have quantified the relative importance of genetic and environmental influence on individual differences in food preferences in adults and children (Reed et al. 1997). A large study of three-year-old British children, suggested moderate heritability for liking of vegetables (54%), fruits (53%) and protein foods (48%), and slightly lower heritability for snacks (29%), starches (32%) and dairy foods (27%) (Fildes, van Jaarsveld, Llewellyn, et al. 2014). Likewise, an earlier study of four year old British twins (Breen et al. 2006) found that variation in liking of fruit (51 %), vegetables (37%), protein foods (78%), and dessert-type foods (20%) all had some genetic basis, albeit with wider confidence intervals for the heritability estimates as expected from the smaller sample size. Importantly, in both studies the environmental influence on variation in food preferences came from aspects of the environment shared by two twins in a family (the 'shared environment', e.g. being raised in the same household), with minimal contribution from environmental influences that are unique to each child (the 'non-shared environment'). This makes sense given the importance of

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[2] Data from this chapter has been published in the American Journal of Clinical Nutrition:
<u>Smith, A.D.</u>, Fildes, A., Cooke, L., Herle, M., Shakeshaft, N., Plomin, R., & Llewellyn, C. (2016) Genetic and environmental influences on food preferences in adolescence. Am. J. Clin. Nutr. 104, 446–453. http://doi.org/10.3945/AJCN.116.133983
*A copy of this paper is provided in **Appendix G2.***

Data from this chapter has been published in the journal Scientific Reports:
<u>Smith, A.D.,</u> Fildes, Forwood, S., A. Cooke, L., and Llewellyn C. (2017) The individual environment, not the family is the most important influence on preferences for common non-alcoholic beverages in adolescence. Scientific Reports. 7(1), pp:16822.
https://www.nature.com/articles/s41598-017-17020-x
*A copy of this paper is provided in **Appendix G3.***

Data from chapter were also presented as a poster on the 4th of November at The Obesity Society's Annual Scientific Meeting (TOS 2016) in New Orleans, USA. The poster won in the category of the 'Top Ten Best Posters in Biobehavioural Obesity Research':
<u>Smith, A.D.,</u> Fildes, A. Cooke, L., Shakeshaft, N., Plomin, R. and Llewellyn C. - Genetic and environmental etiology of non-alcoholic drink preferences in adolescence.
*A copy of this poster is provided in **Appendix D1**.*

the home family environment for the eating behaviour of preschool children (Campbell & Crawford 2001), as the majority of young children's food experiences occur within the family setting (Rosenkranz & Dzewaltowski 2008).

Studies of adult twins have also demonstrated that food preferences tend to have a moderate genetic basis. For instance, a study of habitual dietary patterns in adult female twins (n=3262; mean age: 48.1 (SD=12.80) identified five main dietary patterns that together accounted for 22% of total variance. Overall, all dietary patterns were heritable with genetic influence accounting for 41% of the 'dieting' and 'traditional English' pattern and up to 48% for the 'high alcohol' pattern (Teucher et al. 2007). A further study of older adult twins (n=4640, mean age: 64.7 y. [SD=7.9]) found two dietary patterns to be under low to moderate genetic influence. Heritability for a 'healthy' eating pattern ranged from 33-40 %, and between 15-38% for a dietary pattern characterised by frequent consumption of items high in fat, salt and sugar (van den Bree et al. 1999). However, the unique environment is the most important influence on adult food intake and choice (Hasselbalch et al. 2008; Teucher et al. 2007; van den Bree et al. 1999), with little evidence of any meaningful influence from the shared environment (Keskitalo et al. 2008). This indicates the shared environmental factors that play a role in shaping the development of food preferences in childhood are less important in adulthood, but it is unclear at what stage the influence of the shared environment declines.

At the same time, beverages are increasingly becoming substantial contributors to individual energy intake as the availability and diversity of sugar-sweetened beverages (SSBs), fruit juices and other calorie-containing beverages continues to grow (Popkin & Hawkes 2015). Consumption of SSBs, fruit juices and energy drinks peaks during adolescence (Han & Powell 2013) making this population particularly vulnerable to the detrimental health risks associated with frequent consumption of energy-dense beverages (Popkin 2010; Wang et al. 2008; Ng et al. 2012). Stronger liking for beverages high in energy and decreased liking for beverages low in energy may contribute to variation in intake.

Unlike food preferences, the relative importance of genetic and environmental influences on individual differences in preferences for a variety of non-alcoholic beverages are unknown. Two previous twin studies examined genetic and environmental influences on preferences for coffee (Luciano et al. 2005; Vink et al. 2009), finding moderate genetic influence (42% in Luciano et al, 2005; 62% in Vink et al, 2009). However, both studies defined liking for coffee as relative to liking for tea, indexed as the ratio of number of cups of coffee to tea, consumed per day, rather than absolute liking of coffee per se.

There are no existing studies of the relative influence of genes, and shared and unique environmental factors on food and drink preferences of older adolescents. The aetiology of behavioural traits is known to vary across the lifespan (McGue et al. 1993; Dworkin et al. 1976; Blonigen et al. 2008) and older adolescence is an important developmental transition into adulthood that is characterised by gains in independence. Exploring the relative contribution of genetic and environmental factors to variation in preferences for a range of different types of foods and beverages during older adolescence will help inform where best to direct public health initiatives aimed at decreasing consumption of energy-dense foods and beverages.

Study 1 described the development of a comprehensive food and drink questionnaire which assesses preferences for 63 food items, categorised into six internally reliable food groups, and seven non-alcoholic drink types. This psychometric measure of food and drink preferences will be used in this study to quantify the genetic and environmental influences on variation in preferences for food items and categories, as well as for a variety of non-alcoholic drink types, for the very first time. Alcoholic beverages were not considered for these analyses as previous studies have shown that alcohol drinking behaviours are shaped by moderately to high genetic effects and that these genetic pathways overlap with genetic influences that are associated with risk for alcohol abuse disorders (Schumann et al. 2011; Hansell et al. 2008; Whitfield et al. 2004). Consequently, data collected by the drink preference questionnaire captures both inter-individual variation attributable to liking of the taste of alcoholic beverages as well as the genetic factors that shape alcohol dependence too, which is beyond the scope of this thesis.

5.2 Study aims

This study aims to investigate the relative magnitude of genetic, shared and unique environmental influences on: (i) food preferences; and (ii) non-alcoholic drink preferences in a large sample of older adolescents (18 - 19 years of age).

5.3 Methods

5.3.1 Sample

Study participants were a subsample of twins from TEDS. The sample and recruitment methods are described in detail in Chapter 2. Request to complete the online food preference questionnaires were sent out to the entire sub-sample (3166 pairs; n=6332 individuals); 3155 individual twins consented to participate. Data from twins with serious medical- or perinatal problems or with unknown sex or zygosity were excluded (n=290). Of these, 52 (17.9%) were MZs, 156 (53.8%) were DZs and 82 (28.3%) were of unknown zygosity. Neither self-perceived healthfulness of current diet (a factor conceivably influencing food preferences) (χ^2= 5.918, p= 0.15), food restrictions (χ^2= 0.26, p= 0.87), nor BMI (t=0.45, p=0.65) differed for completer ad non-completers. The final sample consisted of 2865 individuals, which included 1010 monozygotic (MZ), 909 dizygotic same-sex (DZss), and 946 DZ opposite-sex (DZos) individuals. Additionally, data were included from 379 unpaired individuals: 90 unpaired MZs, 107 unpaired DZss and 182 unpaired DZos. Incomplete twin pairs could be included in SEM univariate heritability analyses, as explained in **Chapter 3.**

5.3.2 Measures

5.3.2.1 Anthropometric and sociodemographic measures

A detailed description of age, sex, and zygosity measurements are described in Chapter 3. Briefly summarized, basic demographic information was collected at first contact (age 18 months), including data on date of birth, sex, birth- or medical complications and socioeconomic status. Zygosity of same-sex twin pairs was assigned using a parent-rated similarity questionnaire, validated by DNA analysis. Pairs for whom zygosity was uncertain had their zygosity determined by DNA genotyping, if DNA was available. Participants reported current height and weight, which was used to compute body mass index (BMI), calculated by dividing weight by height squared (kg/m^2).

5.3.2.2 Food and drink preferences

Food and drink preferences were measured using the self-report questionnaire described in Study 1, Chapter 4. Participants rated their liking of 63 individual foods on a 5-point Likert scale, ranging from ‘not at all’ to ‘a lot’; a higher score indicative of greater liking of a food. Participants were instructed to select the ‘not applicable’ option for foods that they had never tried. Twins were asked whether they follow a pescetarian, vegetarian or vegan dietary regimen. Additionally, food allergy information was ascertained using a self-completed food allergies checklist. Food items were grouped into six categories using Principal Components Analysis: vegetables; fruits; meat/fish; dairy; starches; and snacks.

Drink preferences for seven types of beverages were also rated on the same 5-point Likert scale. Preference ratings for milk, tea and coffee were preceded by a question asking: 'Do you drink (or have you ever drunk)...?'. Only participants who responded 'Yes' to this question were subsequently asked how much they liked the drink in question. Participants could select their most preferred milk type from a list of full-fat, semi-skimmed, skimmed or non-dairy milk. Tea and coffee drinkers were asked to indicate whether they preferred their hot beverages sweetened or unsweetened and, with or without milk. The complete food and drink preference questionnaire is shown in **Appendix C1**. Preference test-retest scores for all food and drink items were good over a 2-week period.

5.3.3 Statistical analyses

5.3.3.1 Descriptive statistics

As described in **Study 1**, bivariate correlations were used to compare mean group preferences for all six food preference categories, and for all seven beverage types. Analyses were conducted using SPSS Version 22.0 (SPSS Inc., Chicago, IL, USA).

5.3.3.2 Heritability analyses

Two approaches were used to quantify the relative influence of genetic, shared- and unique-environmental influences on food preference variation.

5.3.3.3 Intra-class correlations

Food item, food category, and drink preference intra-class correlations (ICCs) were calculated for both MZ and DZ pairs, to provide an indication of the pattern of genetic, shared and unique environmental influence on variation in preference scores.

5.3.3.4 Maximum Likelihood Structural Equation Modelling

Maximum Likelihood Structural Equation Modelling (MLSEM) was used to derive precise estimates of the three sources of variation (with 95% confidence intervals), as well as provide goodness-of-fit statistics. Additive genetic factors are denoted by 'A', shared environmental factors by 'C', and unique environmental influences by 'E' (which also includes measurement error).

Initially, food and drink preference scores were residualised for age- and sex-effects. This is a standard procedure in twin modelling because all twins share their age exactly (and sex for same-sex twins), and these factors can therefore inflate the shared environment effect (McGue & Bouchard 1984). First a saturated model was fitted which applies no constraints to the data, and simply estimates means, covariances and variances for MZs and DZs. Then a full ACE model was fitted and compared to the saturated model for goodness-of-fit, as indicated by the Likelihood Ratio Test and the Akaike Information Criterion (AIC).

Non-additive genetic effects, denoted by ‘D’, were also investigated in separate ADE models because the MZ ICCs were greater than twice the DZ ICCs ($ICC_{MZ} > 2\ ICC_{DZ}$) for many of the food and drink preference scores, indicating non-additive genetic effects were contributing to variation. The AIC was used to compare the fit of the two non-nested ACE and ADE models. Sex-limitation models were also tested for each beverage type, to establish if there were sex-specific effects. These models tested whether the magnitude of A, C and E differed for males and females (quantitative sex-differences), and whether the genetic and environmental influences were the same or different for males and females (qualitative sex differences) (Neale et al. 1992).

Because beverage preference scores were skewed, as well as modelling these as continuous scores they were also dichotomized on the median, and modelled using tetrachoric correlation coefficients (TTC’s) (instead of ICCs) and a liability threshold model for categorical (i.e. dichotomized) data.

MLSEM was performed in R (R Core Team 2015), using the structural equation modelling software OpenMx, version 2.2.6 (Boker et al. 2011).

5.4 Results

5.4.1 Summary statistics

Sociodemographic characteristics of the TEDS sample used in this analysis have been described in detail in Chapter 3. A short overview of these, and a summary of dietary characteristics of the subsample are presented in **Table 5.1**. Mean age of the sample was 19.1 years (SD=0.3), and the sample was reasonably lean (mean BMI = 22.3 kg/m^2). 40.2% of participants were male, and the MZ/DZ ratio (MZ pairs = 35.3%) reflected that of the general European twin population (roughly 1:2) (Nussbaum et al. 2015). A small number of study participants reported a vegetarian (n=120; 4.19%), pescetarian (n=77; 2.69%) or vegan diet pattern (n=20; 0.7%). There were few food allergies. Peanut allergy was the most common (n=54; 1.88%), followed by tree nuts (n=34; 1.19%), wheat/gluten (n=31; 1.08%) and dairy (n=28: 0.98%).

Table 5.1 Sociodemographic and dietary characteristics of the study sample (n=2865)

Characteristic	Sample	
Sex [*n* (%)]		
M	1152	(40.2)
F	1713	(59.8)
Zygosity [*n* (%)]		
MZ[1]	1010	(35.3)
DZ[1]	1855	(64.7)
Age [*mean* (SD)]	19.1	(0.3)
BMI [*mean* (SD)]	22.3	(4.2)
Diet type [*n* (%)]		
None	2648	(92.42)
Pescetarian	77	(2.69)
Vegetarian	120	(4.19)
Vegan	20	(0.70)
Food allergy [*n* (%)]		
Peanuts	54	(1.88)
Tree nuts	34	(1.19)
Sesame	5	(0.17)
Dairy	28	(0.98)
Shellfish	13	(0.45)
Fish	6	(0.21)
Egg	4	(0.14)
Wheat/Gluten	31	(1.08)
Soya	5	(0.17)
Celery	2	(0.07)
Mustard	3	(0.10)
Other[2]	49	(1.17)

[1] ‘Other’ includes Strawberries, Oranges and Apples.
[2] *Abbreviations: MZ = Monozygotic; DZ = Dizygotic*

5.4.2 Intraclass correlations for food preferences

Twin correlations for MZ pairs were more similar than DZ pairs for all six food categories suggestive of genetic influence on food preferences (**Table 5.2**). Overall a pattern emerged, showing that DZ within-pair correlations were less than half the MZ ICC’s for all food categories indicating some non-additive genetic influence (D).

Table 5.2 Intraclass cross correlations for preferences for food groups by zygosity (n=2865)

Food item	n[1] (%)[2]	Mean preference score (SD)		MZ[2] ICCs[2] (95% CI)		DZ[2] ICCs[2] (95% CI)	
Vegetables	2865 (100)	3.59	(0.78)	0.58	(0.51, 0.63)	0.17	(0.10, 0.24)
Fruit	2862 (99.9)	4.19	(0.80)	0.52	(0.45, 0.58)	0.23	(0.16, 0.30)
Meat/Fish	2855 (99.7)	3.89	(0.77)	0.45	(0.37, 0.52)	0.18	(0.11, 0.25)
Dairy	2865 (100)	3.62	(0.73)	0.47	(0.40, 0.54)	0.16	(0.09, 0.23)
Snacks	2860 (99.8)	4.39	(0.55)	0.46	(0.39, 0.53)	0.15	(0.08, 0.22)
Starches	2864 (99.9)	3.88	(0.70)	0.36	(0.28, 0.44)	0.08	(0.01, 0.15)

[1] *Abbreviations: ICCs=Intraclass Correlations; MZ=Monozygotic; DZ=Dizygotic*

5.4.3 Intraclass correlations for drink preferences

The MZ and DZ intraclass correlations for the seven non-alcoholic drink types are shown in **Table 5.3**. Drink preference scores showed a similar pattern to food category preference scores, with MZ correlations much higher than DZ correlations for all drink types. Correlations were moderate, with the biggest difference in within-pair resemblance between MZs and DZs observed for milk, suggesting stronger genetic influences on liking for milk compared to other drink types. On average, ICCs for drink preferences were lower (ranging from 0.26 – 0.42 for MZs; 0.00 – 0.22 for DZs) compared to food category ICCs (ranging from 0.36 – 0.58 for MZs; 0.08 – 0.23 for DZs), indicating that environmental factors may play a stronger role in influencing variation in drink preferences than food preferences. Like the ICCs for the food preference category scores, the ratio of MZ/DZ correlations were suggestive of some dominant genetic influence on drink preference scores.

Table 5.3 Drink preference scores and intraclass correlations (ICC) by zygosity

Drink item	n[1] (%)[2]	Mean preference score (SD)	MZ[4] ICC[4] (95% CI)		DZ[4] ICC[4] (95% CI)	
SSB[4]	2841 (99.2)	3.73 (1.37)	0.38	(0.30, 0.46)	0.16	(0.09, 0.22)
NNSB[4]	2827 (98.7)	3.64 (1.34)	0.39	(0.22, 0.38)	0.22	(0.16, 0.29)
Fruit squash	2847 (99.4)	4.23 (1.02)	0.42	(0.34, 0.49)	0.21	(0.14, 0.28)
Orange juice	2849 (99.4)	4.43 (0.97)	0.26	(0.17, 0.35)	0.00	(0.00, 0.05)
Milk	2707 (94.5)	4.22 (0.95)	0.38	(0.29, 0.46)	0.09	(0.01, 0.17)
Tea	2415 (84.3)	4.31 (1.08)	0.53	(0.45, 0.60)	0.00	(0.00, 0.07)
Coffee	1905 (66.5)	3.85 (1.29)	0.34	(0.21, 0.45)	0.07	(0.00, 0.17)

[1] Number of observations included in mean food liking score (excl. observations from individuals that report a restrictive dietary requirement)
[2] Percentage of the full sample that reported trying the item
[3] Preference scores were rated on a 5 point Likert scale, with a higher score indicating a higher preference for the food item.
[4] *Abbreviations: SSB=Sugar-sweetened beverages; NNSB=Non-nutritive sweetened beverages; ICCs= Intraclass Correlations; MZ=Monozygotic; DZ=Dizygotic*

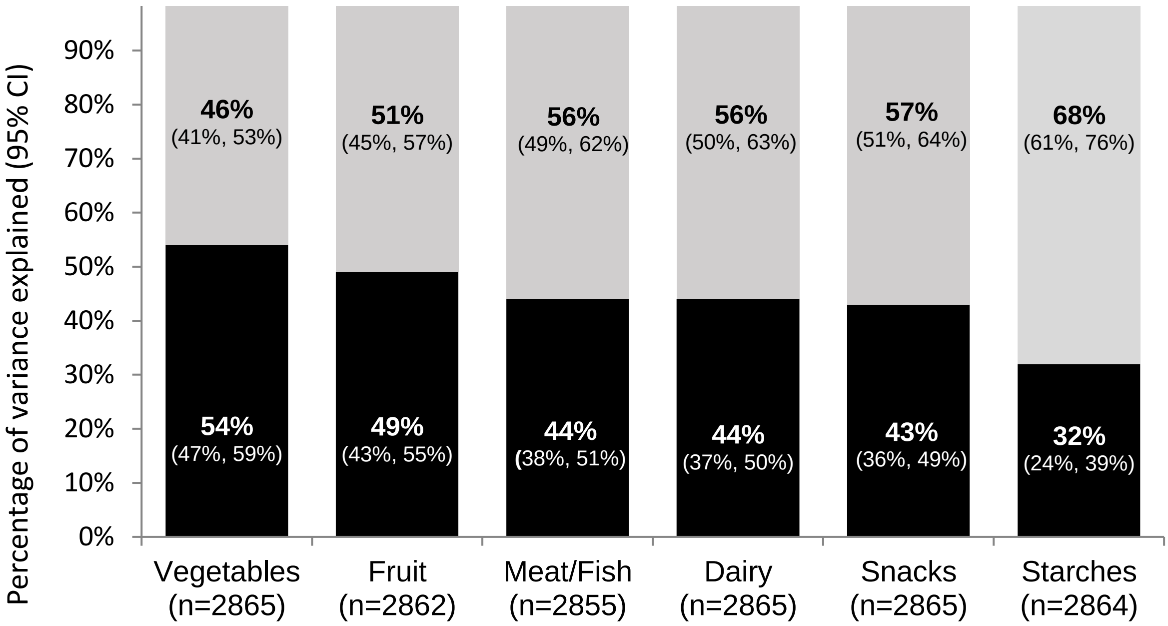
5.4.4 Model-fitting analyses

Results from MLSEM provided more detailed insights into the relative influence of genetic and environmental factors on variation in food and drink preferences.

1.1.1.1 Univariate heritability analyses for food preferences

In general, liking for food categories appeared to be almost entirely explained by genetic influences and unique environmental influences. The best-fitting model for each food group was an AE model (constraining the shared environmental influence (C) to zero). Estimates for all models, with the full model fitting results are shown in **Table 5.4**. Moderate heritability estimates, obtained from ACE models, were found for liking of most food groups: vegetables (0.54; 95% CI: 0.47, 0.59), fruits (0.49; 95% CI: 0.43, 0.55), meat/fish (0.44; 95% CI: 0.38, 0.51), dairy (0.44; 95% CI: 0.37, 0.50), starches (0.32; 95% CI: 0.24, 0.39) and snacks (0.43; 95% CI: 0.36, 0.49). For each of these food groups, approximately half of the observed variation in preference ratings was accounted for by genetic factors. For all of the food groups, unique environmental effects explained remaining variance. The relative proportions of genetic and unique environmental influence on variation in preferences for each of the food groups are shown in **Figure 5.1.** No significant non-additive genetic influences (D) on variation in preferences for any food groups was identified in the ADE models (**Appendix D3**).

Figure 5.1 Genetic and environmental influences for the preference of six food categories



[1] Estimates of the percentage of variance in food preferences explained by genetic (black portions of bars) and environmental (gray portions of bars) factors in 2865 participants from TEDS.

Sensitivity analyses were undertaken to evaluate the impact of self-reported dietary restrictions (e.g. vegetarians or individuals with specific allergies) on ACE estimates for each group. Exclusion of all preference scores for individuals reporting any dietary restriction (n=358) did not alter the results for any food group. Thus, observations from these individuals were only excluded from the analysis if relevant to the reported diet type or allergy (e.g. vegans and vegetarians were not included in the analyses of preferences for meat/fish). Full details of the sensitivity analysis (n=2309) are shown in **Appendix D4.**

Table 5.4 Model fit and parameter estimates for the saturated, ACE model and submodels of food category preferences

Food category	Additive genetic effect (A)	Shared environment effect (C)	Nonshared environment effect (E)	-2LL[3]	Df[3]	AIC[3]	Δ -2LL	p-value
Vegetables[4]								
Sat				6109.386	2856	397.3862		
ACE[1]	0.54 (0.47, 0.59)	0.00 (0.00, 0.04)	0.46 (0.41, 0.52)	6121.434	2859	403.4342	12.048	0.007
AE[2]	**0.54 (0.47, 0.59)**	-	**0.46 (0.41, 0.53)**	**6121.434**	**2860**	**401.4342**	**0**	**1.00**
CE[2]	-	0.32 (0.27, 0.37)	0.68 (0.63, 0.73)	6183.445	2860	463.4448	62.011	<0.001
E[2]	-	-	1.00 (1.00, 1.00)	6316.762	2861	594.7621	195.328	<0.001
Fruit[5]								
Sat				6310.121	2854	602.1213		
ACE[1]	0.49 (0.33, 0.55)	0.00 (0.00, 0.13)	0.51 (0.45, 0.57)	6315.440	2857	601.4399	5.219	0.150
AE[2]	**0.49 (0.43, 0.55)**	-	**0.51 (0.45, 0.57)**	**6315.440**	**2858**	**599.4399**	**0**	**1.00**
CE[2]	-	0.35 (0.30, 0.40)	0.65 (0.60, 0.70)	6344.889	2858	628.8892	29.45	<0.001
E[2]	-	-	1.00 (1.00, 1.00)	6500.514	2859	782.5141	185.074	<0.001
Meat/Fish[6]								
Sat				5864.102	2846	172.1023		
ACE[1]	0.44 (0.34, 0.51)	0.00 (0.00, 0.07)	0.56 (0.49, 0.62)	5870.797	2849	172.7974	6.695	0.082
AE[2]	**0.44 (0.38, 0.51)**	-	**0.56 (0.49, 0.62)**	**5870.797**	**2850**	**170.7974**	**0**	**1.00**
CE[2]	-	0.28 (0.22, 0.33)	0.72 (0.67, 0.78)	5899.942	2850	199.9424	29.145	<0.001
E[2]	-	-	1.00 (1.00, 1.00)	5992.453	2851	290.4527	121.656	<0.001
Dairy[7]								
Sat				6059.620	2855	349.6199		
ACE[1]	0.44 (0.35, 0.50)	0.00 (0.00, 0.06)	0.56 (0.50, 0.63)	6065.078	2858	349.0779	4.542	0.141
AE[2]	**0.44 (0.37, 0.50)**	-	**0.56 (0.50, 0.63)**	**6065.078**	**2859**	**347.0779**	**0**	**1.00**
CE[2]	**-**	0.28 (0.23, 0.33)	0.72 (0.67, 0.77)	6099.114	2859	381.1137	34.036	<0.001
E[2]	-	-	1.00 (1.00, 1.00)	6198.533	2860	478.5330	133.455	<0.001
Snacks								
Sat				4366.211	2856	-1345.79		
ACE[1]	0.43 (0.33, 0.49)	0.00 (0.00, 0.06)	0.57 (0.51, 0.64)	4379.694	2859	-1338.31	13.429	0.004
AE[2]	**0.43 (0.36, 0.49)**	-	**0.57 (0.51, 0.64)**	**4379.694**	**2860**	**-1340.31**	**0**	**1.00**
CE[2]	-	0.27 (0.22, 0.32)	0.73 (0.68, 0.78)	4410.221	2860	-1309.78	30.527	<0.001
E[2]	-	-	1.00 (1.00, 1.00)	4498.200	2861	-1223.80	118.506	<0.001
Starches[8]								
Sat				5848.940	2856	136.9400		
ACE[1]	0.32 (0.23 0.39)	0.00 (0.00, 0.05)	0.68 (0.61, 0.76)	5861.103	2859	143.1027	12.163	0.007
AE[2]	**0.32 (0.24, 0.39)**	-	**0.68 (0.61, 0.76)**	**5861.103**	**2860**	**141.1027**	**0**	**1.00**
CE[2]	-	0.18 (0.13, 0.24)	0.82 (0.76, 0.87)	5881.719	2860	161.7192	20.616	<0.001
E[2]	-	-	1.00 (1.00, 1.00)	5922.439	2861	200.4394	61.336	<0.001

Maximum Likelihood Structural Equation Modelling (MLSEM) was used to derive estimates of A, C and E, as well as provide two goodness-of-fit statistics; -2LL and the AIC respectively. The selection of the most parsimonious model was indicated by the p-value and the lowest absolute value of the AIC·

[1] **The full ACE model was nested within the saturated model**

[2] Sub-models were nested within the full ACE model

[3] *Abbreviations; - 2LL= -2 times log-likelihood of data, df= degrees of freedom, AIC= Akaike Information Criterion*

[4] excludes observations for celery liking from individuals self-reporting an allergy against celery.

[5] excludes observations for strawberries, apples and oranges for individuals self-reporting a strawberry, apple or orange allergy.

[6] excludes observations for all meat items from self-reported pescetarians, vegetarians and vegan. White fish, oily fish, tinned tuna and smoked salmon liking includes pescetarians' observations but excludes preference scores from individuals reporting a fish allergy

[7] excludes observation for egg liking from individuals reporting an egg allergy and vegans. Food preference scores for soft cheese, hard cheese, butter, cream, yoghurt, cottage cheese and custard were excluded from vegans and individuals self-reporting a dairy allergy.

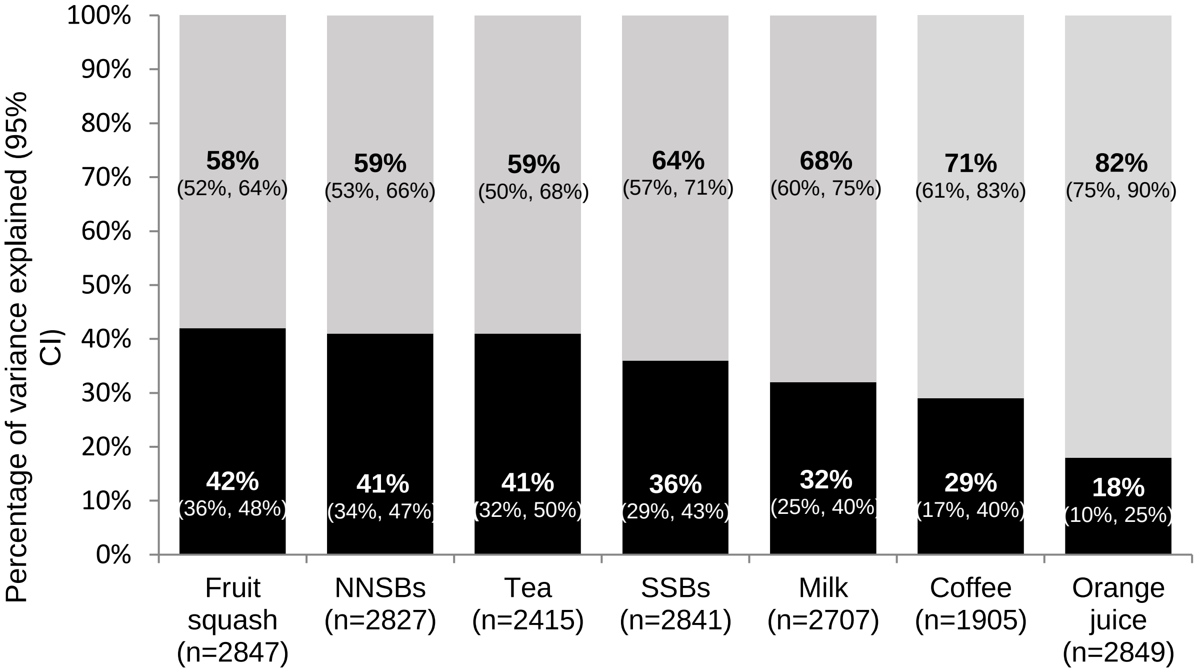
[8] excludes observations for wheat cereal from individuals reporting a wheat/gluten allergy.

In keeping with the results for the food groups, for all of the *individual* food items, it was possible to drop the shared environmental factor (C), with AE models being preferred in every case. In fact, for almost all individual foods the shared environmental effect was estimated to be 0, indicating no detectable effect of the shared environment on any food preferences in this sample. Heritability estimates for *individual* food items ranged from 0.18 (95% CI: 0.10, 0.25) for bread, to 0.53 (95% CI: 0.46, 0.59) for avocado. The ACE modelling results for each individual food item are presented in full in **Appendix D2.**

1.1.1.2 Univariate heritability analyses for drink preferences

The relative contributions of genetic and environmental influences on individual differences in drink preferences are shown in **Figure 5.2**. Similar to food category preferences, there was no significant influence of the shared environment on individual differences for liking of various drink categories.

Figure 5.2 Genetic and environmental influences for the preference of seven non-alcoholic beverages



[1] Estimates of the percentage of variance in beverage preferences explained by genetic (black portions of bars) and environmental (grey portions of bars) factors in 2865 participants from TEDS.

Heritability estimates (A) across all beverage types were moderate to low for each of: fruit squash (0.42; 95% CI: 0.36, 0.43), NNSBs (0.41; 95% CI: 0.34, 0.47), tea (0.41; 95% CI: 0.32, 0.50), SSBs (0.36; 95% CI: 0.29, 0.51), milk (0.32; 95% CI: 0.25, 0.40), coffee (0.29; 95% CI: 0.17, 0.40), and orange juice (0.18; 95% CI: 0.10, 0.25). The 95% confidence intervals demonstrated that genetic influences were significantly higher for liking of SSBs, NNSBs, tea and fruit squash, than for liking of orange juice. No significant influence of the shared environment was observed for liking of any of the beverages, with the remaining variance being explained by environmental effects unique to each individual twin. AE models (that dropped the C component of variance) were therefore preferred for each beverage type. The relative contribution of genetic and environmental influences on variation in preferences for each drink type and the ACE model results with goodness-of-fit statistics are shown in **Table 5.5.**

Table 5.5 Model fit and parameter estimates for the saturated, ACE model and submodels of beverage preferences

Beverage type	Additive genetic effect (A)	Shared environment effect (C)	Nonshared environment effect (E)	-2LL[3]	Df[3]	AIC[3]	Δ -2LL	p-value
SSBs[3]								
Sat				9609.825	2832	3945.825		
ACE[1]	0.36 (0.26, 0.43)	0.00 (0.00, 0.09)	0.64 (0.57, 0.71)	9614.843	2835	3944.843	5.018	0.170
AE[2]	**0.36 (0.29, 0.43)**	**-**	**0.64 (0.57, 0.71)**	**9614.843**	**2836**	**3942.843**	**0**	**1.00**
CE[2]	-	0.24 (0.19, 0.29)	0.76 (0.71, 0.81)	9631.547	2836	3959.547	16.704	<0.001
E[2]	-	-	1.00 (1.00, 1.00)	9703.572	2837	4029.572	72.025	<0.001
NNSBs[3]								
Sat				9545.841	2818	3909.841		
ACE[1]	0.35 (0.15, 0.47)	0.05 (0.00, 0.20)	0.60 (0.55, 0.68)	9546.322	2821	3904.322	0.481	0.923
AE[2]	**0.41 (0.34, 0.47)**	**-**	**0.59 (0.53, 0.66)**	**9546.719**	**2822**	**3902.719**	**0.397**	**0.529**
CE[2]	-	0.28 (0.23, 0.33)	0.72 (0.67, 0.77)	9557.576	2822	3913.576	11.254	<0.001
E[2]	-	-	1.00 (1.00, 1.00)	9660.699	2823	4014.699	114.38	<0.001
Orange juice								
Sat				7844.431	2840	2164.431		
ACE[1]	0.18 (0.09, 0.25)	0.00 (0.00, 0.04)	0.82 (0.75, 0.90)	7862.541	2843	2176.541	18.11	<0.001
AE[2]	**0.18 (0.10, 0.25)**	**-**	**0.82 (0.75, 0.90)**	**7862.541**	**2844**	**2174.541**	**0**	**1.00**
CE[2]	-	0.08 (0.03, 0.14)	0.92 (0.86, 0.97)	7873.266	2844	2185.266	10.725	0.001
E[2]	-	-	1.00 (1.00, 1.00)	7881.523	2845	2191.523	18.982	<0.001
Fruit squash								
Sat				8031.215	2838	2355.215		
ACE[1]	0.42 (0.23, 0.48)	0.00 (0.00, 0.15)	0.58 (0.52, 0.90)	8034.727	2841	2352.727	3.512	0.319
AE[2]	**0.42 (0.36, 0.48)**	**-**	**0.58 (0.52, 0.64)**	**8034.727**	**2842**	**2350.727**	**0**	**0.998**
CE[2]	-	0.29 (0.24, 0.34)	0.71 (0.66, 0.76)	8051.672	2842	2367.672	16.945	<0.001
E[2]	-	-	1.00 (1.00, 1.00)	8157.514	2843	2471.514	122.79	<0.001
Milk								
Sat				7269.105	2698	1873.105		
ACE[1]	0.32 (0.25, 0.40)	0.00 (0.00, 0.06)	0.68 (0.60, 0.75)	7281.350	2701	1879.350	12.245	0.007
AE[2]	**0.32 (0.25, 0.40)**	**-**	**0.68 (0.60, 0.75)**	**7281.350**	**2702**	**1877.350**	**0**	**1.00**
CE[2]	-	0.20 (0.14, 0.26)	0.80 (0.74, 0.86)	7298.526	2702	1894.526	17.176	<0.001
E[2]	-	-	1.00 (1.00, 1.00)	7340.874	2703	1934.874	59.524	<0.001
Tea								
Sat				7098.183	2406	2286.183		
ACE[1]	0.41 (0.32, 0.50)	0.00 (0.00, 0.03)	0.59 (0.50, 0.68)	7134.002	2409	2316.002	35.82	<0.001
AE[2]	**0.41 (0.32, 0.50)**	**-**	**0.59 (0.50, 0.68)**	**7134.002**	**2410**	**2314.002**	**0.00**	**1.00**
CE[2]	-	0.19 (0.12, 0.26)	0.81 (0.74, 0.88)	7171.829	2410	2351.829	37.83	<0.001
E[2]	-	-	1.00 (1.00, 1.00)	7201.412	2411	2379.412	67.41	<0.001
Coffee								
Sat				6351.94	1896	2559.94		
ACE[1]	0.29 (0.12, 0.39)	0.00 (0.00, 0.11)	0.71 (0.61, 0.83)	6358.921	1899	2560.921	6.9809	0.07
AE[2]	**0.29 (0.17, 0.40)**	**-**	**0.71 (0.61, 0.83)**	**6358.921**	**1900**	**2558.921**	**0**	**1.00**
CE[2]	-	0.17 (0.09, 0.25)	0.83 (0.75, 0.91)	6366.625	1900	2566.625	7.7045	<0.001
E[2]	-	-	1.00 (1.00, 1.00)	6382.128	1901	2580.128	23.208	<0.001

Maximum Likelihood Structural Equation Modelling (MLSEM) was used to derive estimates of A, C and E, as well as provide two goodness-of-fit statistics; -2LL and the AIC respectively. The selection of the most parsimonious model was indicated by the p-value and the lowest absolute value of the AIC.

1 The full ACE model was nested within the saturated model

2 Sub-models were nested within the full ACE model

3 Abbreviations: - *2LL= -2 times log-likelihood of data, df=degrees of freedom, AIC=Akaike Information Criterion, NNSBs= Non-nutritive sweetened beverages, SSB=Sugar-sweetened beverages*

Because the MZ ICCs were greater than twice the DZ ICCs for SSBs, orange juice, milk, tea, and coffee preference scores, suggestive of some non-additive genetic effects, ADE models were also examined and compared to ACE models (**Appendix D6**). For liking of orange juice, milk and tea, the ADE model provided a better fit than the ACE model (ΔAIC= -7.8, -4.07, and -24.19, respectively), but no statistically significant non-additive genetic effects (D) were found. AE models therefore provide the most parsimonious solutions across all beverage types. Similarly, because scale scores were skewed for orange juice, milk, tea and coffee, threshold models (treating beverage preference scores as binary traits) were considered as well as the models of continuous data. However, the genetic and environmental aetiology estimates from the threshold models did not differ significantly from the results shown in **Table 5.3**. Due to the loss in statistical power to estimate genetic variance components accurately when a trait is modelled as a binary rather than a continuous variable, results from the liability threshold models are shown in **Appendix D7 and D8.** Similarly, results from the sex-limitation models found no strong evidence of significant sex differences. Parameter estimates and goodness-of-fit statistics for the full qualitative and quantitative sex-limitation models are also shown in **Appendix D9 – D16.1.**

5.5 Discussion

5.5.1 Summary of findings

This study establishes for the very first time, the relative importance of genetic, shared and non-shared environmental influences on variation in preferences for a large number of individual food items, food groups, and drinks, in late adolescence. The results demonstrate that shared environmental factors common to both siblings, e.g. the household or school setting, did not appear to significantly influence food preferences during late adolescence. Nonetheless, in keeping with previous paediatric and adult studies, moderate genetic influences on food preferences were observed in this sample. Like food preferences, variation in drink preferences also tended to have a moderate genetic contribution in older adolescents.

5.5.2 Heritability of food preferences

These findings confirm previous research in children and young adults which consistently show sizeable genetic influence on individual variation in food preferences or intake (Keskitalo et al. 2008; Breen et al. 2006; Fildes, van Jaarsveld, Llewellyn, et al. 2014). Importantly, the significant shared environmental influences observed in childhood (Breen et al. 2006; Fildes, van Jaarsveld, Llewellyn, et al. 2014) appear to have been replaced entirely by non-shared environmental factors by the time individuals enter older adolescence and start to make the transition into early adulthood.

Moderate heritability of food preferences in this sample is in line with the results of the only study that has investigated genetic and environmental influences on dietary intakes in a sample of young adults (Keskitalo et al. 2008). For older adolescents, food encounters increasingly occur outside of the family home; finding no influence of the shared environment variation in food and drink preferences may therefore reflect this natural transition. The absence of an enduring shared environmental effect (i.e. from earlier on in life) has also been documented for other dietary behaviours, e.g. food intake patterns (Heitmann et al. 1999; van den Bree et al. 1999) and general nutrient-intake (Hur et al. 1998; de Castro 1998; Heller et al. 1988). While these findings are broadly consistent with most food preference research undertaken in adults, a small Danish study of adults twins did find significant influences of the shared environment on dietary intake (Hasselbalch et al. 2008). However, these estimates were derived from actual food intake data (not food preferences) collected using 1-month dietary recalls, which are known to be inaccurate, and had wide 95% confidence intervals. Because dietary recall methods for dietary intake are likely to be affected by inaccuracies such as underreporting or altered food intake when participants know their diet will be assessed by researchers, people may well be better at reporting general likes and dislikes rather than the specific foods they have consumed (which they might forget and also fluctuates from day to day or week to week) (Shim et al. 2014)

The findings of substantial genetic influence on variation in food preferences replicated results from a previous study in three to four year old twins (Fildes, van Jaarsveld, Llewellyn, et al. 2014). This suggests that food preferences emerge early in life, can be reliably measured and are influenced by similar levels of genetic influences over time. The similarity of the food preference heritability estimates across the different food categories compared to the Fildes et al. (2014) study can be seen in **Appendix D5**. While these results suggest the influence of shared environmental effects detected in early childhood may disappear by late adolescence, the genetic and environmental influences on these traits have not been studied in the same sample at both ages. It is therefore possible that the different estimates of the influence of the shared environment on food preferences could reflect other factors such as cohort effects. For instance, the twins of the Gemini twin cohort in the Fildes et al (2014) study were born into a more obesogenic environment (year of birth=2007) participants of the TEDS cohort were all born from 1994 to 1996. In a recent pooled analysis of NHANES data from the US, there some suggestion of a statistically significant cohort effect whereby cohorts born later had an increased risk of obesity, even after adjustment for age and time period (Keyes et al. 2010). This provides some indication that a cohort effect may be one mechanism influencing differences between family food environments and general obesogenic pressures which may have contributed to the larger shared environment effect in the Gemini twins (relative to when the TEDS twins were little). A further key reason could be that in Gemini the same person rated preferences for both twins, but in TEDS the two twins themselves reported their liking separately. Common rater bias could also account for the shared environmental effect

It is well-established that sweet tastes are universally accepted, and bitter tastes disliked (Mennella 2014). These dispositions are thought to be artefacts of an evolutionary adaptive process facilitating identification of safe sources of dietary energy and avoidance of potentially toxic substances (Breslin 2013). However, there is considerable population variation in these preferences and there is now some molecular genetic evidence to support the heritability estimates observed for variation in some food preferences. Polymorphisms in the TAS2R genes, a family of 25 bitter taste receptors, have been associated with variation in sensitivity towards bitter-tasting compounds such as Phenylthiocarbamide (PTC) (Turner-McGrievy et al. 2013) and 6-n-propylthiouracil (PROP) (Fox 1932; Boxer & Garneau 2015). Individuals with a copy of the dominant PAV haplotype in the TAS2R38 gene are most sensitive to PROP (Boxer & Garneau 2015), and a number of studies have associated lower liking of green or cruciferous vegetables with this genotype (Dinehart et al. 2006; Duffy et al. 2010; Bell & Tepper 2006; Colares-Bento et al. 2012; Keller et al. 2002; Drewnowski et al. 1999; Turnbull & Matisoo-Smith 2002) or grapefruit and grapefruit juice (Drewnowski et al. 1997; Tepper et al. 2003). Nevertheless, evidence for this genotype as an important influence on the development of fruit and vegetable consumption patterns is not unequivocal (Tepper et al. 2009). There are some studies that have documented null associations between the taster genotype of TAS2R38 and brassica vegetable intake (Gorovic et al. 2011), aversion to vegetables (Ooi et al. 2010), green vegetable intake frequency (Timpson et al. 2005; Baranowski et al. 2011), or bitter vegetable liking or intake (Feeney et al. 2014; Anliker et al. 1991). Evidently, more research is needed to identify genetic variants associated with other taste preferences, such as sweet preference. In a large recent GWAS, 15 SNPs were identified that explained significant variation in liking for 20 specific foods, across four broad food groups (Pirastu, Kooyman, Traglia, et al. 2016). Loci associated with the ‘vegetable’ group, related to variation in the liking of artichokes, broccoli, chicory and mushrooms. For ‘fatty foods’, loci were associated with variation in preference scores for bacon and ‘butter or oil on bread’, whilst preferences for ‘dairy foods’ were associated with loci that related to liking of blue cheese, ice cream or yoghurt. Loci associated with the ‘bitter foods’ group, explained variation in the preference rating of dark chocolate, coffee, and liver. However, there may also be psychological traits that underlie food preferences. For instance, a recent study of 3-year old British twins established that a substantial proportion of the genetic influence on fruit and vegetable liking could be explained by the genetic influence on food fussiness (Fildes, Llewellyn, et al. 2014).

Other genetic variants have been identified that are associated with variation in taste perception, but their modes of action are largely unknown. Possible mechanisms include: taste receptor density on the tongue (Bartoshuk et al. 1994); reward circuitry (Schoenfeld et al. 2004); and cognitive processes related to self-regulation (Steinle et al. 2002), extraversion (Knaapila et al. 2011), food neophobia (Cooke et al. 2007) or anxiety (Platte et al. 2013) all of which have been associated with food preferences (Grimm & Steinle 2011). Along the same lines, the evidence for considerable genetic influences on differences in drink preferences is unsurprising. Some of the beverages assessed (SSBs, NNSBs, fruit squash, orange juice) were sweet, and there is a growing body of literature implicating genetic variants in individual differences in sweetness sensitivity, and for differential sensitivity to various artificial sweeteners. A recent twin study by Hwang et al (Hwang et al. 2015) documented a shared genetic pathway underlying the sensitivity of sugars and artificial sweeteners. Up to half of the phenotypic variation in liking and intake frequency of sweet foods was attributable to genetic factors in a different study (Keskitalo, Tuorila, et al. 2007), and it has been suggested that genes located on Chromosome 16 might be important sources of this genetic variability (Keskitalo, Knaapila, et al. 2007).

5.5.3 Heritability of drink preferences

This study established for the very first time that preferences for various non-alcoholic drinks, like foods, are entirely under the influence of genetic- and non-shared environmental factors in older adolescents. This suggests that shared environmental factors are unimportant in shaping preferences for non-alcoholic beverages in this age group.

Similar to previous studies on the aetiology of food preferences, variation for the liking of seven beverage types was found to have a small to moderate genetic basis. Two previous studies have investigated the genetic and environmental contributions to *relative* liking for coffee (over tea), showing that approximately 42% to 62% of variation in coffee preference was attributable to genetic differences between adults (Luciano et al. 2005; Vink et al. 2009). These estimates are at the higher end of those observed in the current study (ranging from 18% for orange juice, to 29% for coffee, and up to 42% for fruit squash). The estimates for relative coffee preference are perhaps best compared to the estimate for the liking of coffee, however it is important to reiterate that direct comparison to the two previous studies is limited because the studies by Luciano et al (2005) and Vink et al (2009) characterised liking for coffee by making a direct comparison between coffee and tea intake frequency. The preference ratings for coffee likely encompass genetic sensitivity to, and enjoyment of, the stimulation provided by caffeine. However, not only coffee but many other carbonated fizzy beverages contain caffeine, a psychoactive substance that may be a key driver of beverage liking. Gareth and Griffiths (1998) suggested the liking for fizzy caffeinated beverages may be reinforced by the invigorating effect of caffeine, and avoidance of withdrawal symptoms, rather than an affinity for the actual flavour of the beverage (Garrett & Griffiths 1998). These studies suggest that both direct genetic variability in the biological caffeine-response (e.g. polymorphisms in the caffeine metabolism pathway), and the more indirect psychological mechanisms (e.g. sensitivity to caffeine-induced anxiety) might be influencing caffeinated beverage preference scores (Yang et al. 2010).

In line with the heritability estimates for SSBs (36%), NNSBs (41%), and fruit squash (42%) observed in the present study, one previous twin study showed variation in liking for 'sweet and high carbohydrate foods' which included diet and non-diet soft beverages and fruit juices, to be moderately heritable (52%) in a large sample of adults (n=2596) (Pallister et al. 2015). However, this previous study only provides limited comparability due to sweet foods and beverages being combined in this category.

Sweetness dominates as the underlying taste of SSBs, NNSBs, fruit squash, orange juice, and to some extent for the natural sweetness of milk. Finding moderate genetic influence on liking for non-alcoholic beverages may in part reflect genetically-determined sensitivity to or preference for sweetness. A previous twin study found that sweetness sensitivity for two different sugars (glucose and fructose) and two different non-nutritive sweeteners (aspartame and neohesperidine dihydrochalcone), was largely explained by a common genetic pathway underlying them all; this suggests a genetically-determined mechanism which may influence variation within the innate liking for sweet flavours in general (Hwang et al. 2015). In addition, results from a study of 26 Finnish families (n=146 individuals; 18-78 yrs.) reported moderate heritability estimates for pleasantness ratings for a strong sweet solution (41%), the pleasantness rating (40%) and frequency of consumption (50%) of sweet foods, and craving for sweet foods (31%) (Keskitalo, Knaapila, et al. 2007). To date no genome-wide association study has been undertaken to identify common genetic variants associated with variation in sweetness preference. This is likely the result of the limited availability of data on sweetness preference measures in large samples with genome-wide genotyping. One GWAS study in two Japanese population samples (n=5430) did however identify a single SNP in intron 1 of the ADIPOQ gene to be significantly associated with greater frequency of confectionary consumption. Furthermore, polymorphisms in the sweet taste receptor genes T1R2 and T1R3 have been associated with variation in sugar consumption (Eny et al. 2010) and sucrose taste sensitivity (Fushan et al. 2009). Evidence from a mouse study suggests sweetness perception is reliant on the functionality of taste receptor subunits T1R1/T1R3. Zhao et al (2003) observed that mice knockout models for these genes fail to respond to stimulation by sweetness (Zhao et al. 2003), implicating these genes as potential contributors to the observed genetic basis of sweet-beverage preference scores.

The questions relating to tea and coffee preference in this study specifically enquired about unsweetened varieties of these drinks, meaning these were the only non-sweet beverages considered in the current analyses. Evidence from a recent twin study (n=1901; mean age: 16.2 y) found considerable correlations (r= 0.35-0.40) between the mean perceived intensity of a “sweet factor” (comprising intensity ratings for glucose, fructose, neohesperidine dihydrochalcone, and aspartame) and perception intensity of three bitter solutions (sucrose octa-acetate, quinine, and caffeine). The correlations were largely explained by shared genetic influences, with 8% of sweetness perception and 17-37% of the bitterness perception ratings attributable to genes common to both traits. In addition, the magnitudes of the heritability of sweetness perception (36%) and of bitterness perception (35-40%) were similar (Hwang et al. 2016). These findings support our results showing heritability estimates did not differ significantly between sweet beverage types (18-42% for SSBs and NNSBs) and non-sweet types (29-41% for tea and coffee). However, it is important to note that the sample sizes were slightly lower for coffee and tea in the present study (n=2415 for tea, n=1905) for coffee), as mean scores for this group only included participants who reported drinking these hot beverages. This may have influenced the heritability estimates for the liking of these non-sweet beverages.

Additionally, it is important to consider that coffee and tea are among the most commonly consumed caffeine-containing beverages worldwide. While caffeine is often consumed for its stimulating affects, it can also induce anxiety in individuals with habitually low caffeine intake levels. Particularly the TT allele within the ADORA2A gene (rs5751876) has been linked to greater caffeine-induced anxiety, which hints at further genetically influenced pathways that may be upstream factors shaping the liking for beverages such as tea and coffee (Alsene et al. 2003). Nevertheless, this single nucleotide polymorphism (SNP) in itself cannot account for the observed moderate heritability estimates for tea and coffee (29-41%, respectively). Other heritable factors remain to be identified. This was recently emphasized in a RCT (n=318) in which habitual caffeine consumers were randomized to a placebo or caffeine pill and observed for symptoms of withdrawal (alertness, anxiety and headache) over a 24h period. Overall the trial found evidence suggesting that variation in the anxiogenic effect of caffeine is associated with the ADORA2A rs5751876 SNP yet that this variation in response does not prevent individuals from regularly consuming caffeinated beverages (Rogers et al. 2010).

Variation in preferences for beverages was influenced strongly by individual environmental factors (e.g. friends, lifestyle choices), and less so by genetic factors (shared environmental factors were undetectable). This was especially the case for orange juice, coffee and milk, for which 82%, 71%, and 68% of variation in preference scores were explained by non-shared environmental factors, respectively. Similarly to the findings for food preferences, the strong influence of the non-shared (versus shared) environment on variation in drink preferences likely reflects the lifestyle changes experienced by adolescents, with greater time spent interacting with peers outside the home, and conforming to perceived societal- and peer pressures. In this respect, (social) media and the commercial food and beverage environment may start to replace family rules and habits (learned at home), exerting a stronger influence on food and beverage choices.

5.5.4 Environmental influences on food and drink preferences

People intuitively think of cultural influences as playing an important role in shaping food and drink preferences. Many of these cultural influences - both those at the family level, as well as the wider societal level, such as national cuisines and drinks – are shared by twin pairs. Finding a substantial influence of the non-shared environment on food and drink preferences could therefore be considered surprising if it would be expected that the cultural environment in childhood would still influence dietary preferences in young adults. However, this observation suggests that twin pairs respond differently to the environmental influences they are both exposed to – e.g. most people living in the UK are exposed to tea, but it is not uniformly liked by all. This supports a wealth of research highlighting that the non-shared environment is often the dominant influence on older children for many traits, which suggests that children growing up within the same family experience the same environmental exposures differently (Plomin 2011).

While older adolescents mature and their proximal relationships with friends and family evolve, it is important to consider that these changes occur within the wider societal context. For instance, indicators of lower SES have previously been identified as determinants of higher SSB intake and intake of dietary energy from sweetened beverages, overall (Han & Powell 2013; Paulsen et al. 2016). The association between lower SES and higher SSB consumption has been demonstrated in high-income countries such as the UK and the US (Han & Powell 2013; Vereecken et al. 2005). No equivalent study has yet investigated the influence of SES on a comprehensive range of non-alcoholic drink preferences. In our study, we found no effect of the shared environment on preferences for a number of different non-alcoholic drink categories but this does not mean that SES is unimportant. As previously suggested in an elegant piece of work by Plomin (2011), growing up in a shared household may be experienced differently by each individual living in that same household. As such, the unique experience of SES may be contributing to the substantial non-shared environmental influence on beverage preferences seen in our study, even though SES intuitively may be considered a shared-environmental factor (Plomin 2011).

The sizeable non-shared environmental influence may in-part be a consequence of the strong influence of the pervasive food environment on dietary intake (Caspi et al. 2012; Black et al. 2014). While the effect on food and drink intake may be more direct, there is a plausible knock-on effect on food and drink preferences too. Given some of the twin pairs will now be living separately or attending different workplaces/colleges, availability and food purchasing outlets near the home, the workplace, as well as readily accessible during a commute, strongly shape individual food intake habits (Kestens et al. 2010; Lebel et al. 2017). If people live in a ‘food desert’ or ‘junk food jungle’, this very much influences the categories of foods they purchase, especially impacting access to fresh fruit and vegetables. Evidence for the effect of environmental exposure to food outlets close to home, at work, and along commuting routes on dietary intake has been recently documented in a population-based study in the UK. In this study of 5442 adults, it was shown that individuals highly exposed to takeaway food outlets, on average consumed an additional 5.7g of fast food per day compared to those least exposed (equivalent to a 15% higher consumption of fast food) (Burgoine et al. 2014). Over the course of two weeks, this difference equates to a small serving of fast food fries (71 g; 229 kcal), which is a demonstration of the substantial environmental contribution to fast food intake in the UK (US Department of Agriculture 2017).

During adolescence, individuals likely begin to diversify the food and drinks they consume, while developing an appreciation for more complex flavours (e.g. the bitterness in alcoholic beverages or vegetables) (Cowart et al. 1994). Interestingly, variation in beverage preferences appeared to have a slightly higher environmental influence than variation in food preferences, measured in this sample at the same age. For instance, the heritability for the preference of fruit (49%), a category which included the liking for oranges, was much higher than the heritability for the liking of orange juice (18%). Preferences for texture, intensity of sweetness, and effort involved in consumption are associated with food choice (Mojet & Köster 2002), and are likely to have a strong genetic component to their expression. Previous studies have demonstrated that variation in sensitivity for these food attributes contributes to fussy eating behaviours (Werthmann et al. 2015; Johnson et al. 2015; Dovey et al.). Preferences for drinks are thus less influenced by these genetic determinants of food acceptance and perhaps more related to the environmental context in which people might consume these non-alcoholic drinks. This may have been the case for orange juice and milk, with 82% (95% CI: 75%, 90%) and 68% (95% CI: 60%, 75%) of variation in their preference scores explained by non-shared environmental factors, respectively. Milk and orange juice are commonly considered healthy drinks, and good sources of protein (in milk) and vitamin C (in orange juice). Both are also cheaper than other dietary sources that may be considered healthy sources of these nutrients (e.g. fresh meat or fresh fruit). It is possible that the high non-shared environmental influence on preference for specifically these drink types may in part be explained by individuals frequently opting for these drinks due to personal financial constraints to supplement their diets, or for other health-conscious reasons, and as a result this may have influenced liking for these drinks (Drewnowski & Rehm 2015).

Alternatively, both orange juice (45 kcal/100 ml) and milk (35-68 kcal/100 ml) are quite calorific, and therefore might be influenced by whether people (or those around them) are engaging in restrictive or weight control behaviours. Unfortunately, no data on these behaviours were collected in TEDS. Then again, there are many other important factors that may influence how often an individual may purchase a beverage, which affects the development of preference for a certain drink. The need to keep orange juice and milk refrigerated (once opened), coupled with their limited shelf-life conceivably influence purchase frequency. SSBs, NNSBs, fruit squash, tea, and coffee in contrast can be stored for longer periods of time. Distance to affordable supermarkets, how good you are at remembering to buy certain food or drink types regularly, and access to a fridge are further salient determinants of how frequently these drinks may be purchased. These factors all likely contribute to the non-shared environmental context shaping drink preferences for non-alcoholic beverages.

Perhaps more importantly, our results indicate that beverage preferences may have even greater potential for environmental modification than food preferences. This is encouraging given the concern regarding high intake of energy dense and sweetened beverages such as SSBs, and may reflect the effective marketing strategies of SSB manufacturers in the UK. The potent effect of advertising on children's consumption of SSB's was recently revealed in study of 9760 school children (10-11 years) in the US. On average, exposure to 100 TV advertisements for SSBs was significantly associated with a 9.4% increase in the child's consumption of SSBs over a three-year period (Andreyeva et al. 2011). However, other macro-level factors such as SES and the precise mechanism by which these determinants affect SSB intake and preferences will require more research if environmental intervention is to shift drink choices towards healthier options.

Nevertheless, substantial heritability of food and drink preferences does not preclude the potential for environmental modification, especially when considered alongside the sizeable environmental influence observed in the present study. Experimental research has demonstrated that repeated exposure to tastes increases flavour acceptance, showing that environmental modification is possible (Cooke et al. 2011; Corsini et al. 2013; Remington et al. 2012; Holley et al. 2015; Fildes, van Jaarsveld, Wardle, et al. 2014). At the population level, nation-wide salt reduction strategies have provided evidence of the plasticity of adult taste preferences. Since 2003, the UK Food Standard Agency's reformulation program, has sustained efforts to drive gradual reduction of sodium-levels in the UK food supply (Wyness et al. 2012). Over the course of 7 years, average salt intake was successfully lowered by 15%, and consumers were largely unaware of the gradual reduction of salt levels in their food supply. This voluntary salt reduction program was successful in drastically cutting salt intake in the UK, and provides evidence for the malleability of adult salt preference, directly attributable to alteration of total sodium intake of total dietary intake (He et al. 2014). No research has yet investigated the effectiveness, acceptability or feasibility of a targeted food or drink preference modification program in an adolescent or adult population.

5.5.5 Strengths and limitations

The observation that shared environmental influences on food preferences from childhood appear to be replaced by non-shared environmental factors by the time individuals enter older adolescence were based on cross-sectional data. Because heritability estimates are time- and population-specific, future longitudinal data from the same sample are needed to test the assumption that shared environmental influences disappear once individuals are able to make autonomous dietary choices. Such data will be key in establishing whether the effect of the early shared family experience relating to food and drink preferences only temporarily dips in late adolescence, or is completely displaced by adulthood.

Although the twin design can help quantify the relative contribution of genetic and environmental factors to variation in food and drink preferences, it cannot identify the specific genes, or environmental factors involved. Liking scores for popular food and drink items such as chocolate and orange juice were high, with mean scores less than 1 SD below the maximum, which may have limited estimates of the relative importance of genetic or environmental influences on food and drink preferences (Reed et al. 1997). Nonetheless, the 5-point Likert scale was able to capture sufficient variance to allow SEM modelling, and the food and drink items with possible ceiling effects didn't show significantly different results from the others. Although this is the first study to examine the aetiology of a range of non-alcoholic beverage preferences, only a limited number of beverage types were included in the questionnaire. It is also worth pointing out that the beverages were sometimes treated as categories (e.g. 'SSBs', 'NNSBs'), while others were asked about as single drinks (e.g. 'orange juice' and 'fruit squash') – whereas tea and coffee are arguably somewhere in the middle as there are multiple variants of each. This is not as robust as the multiple item food preference questionnaire which provided the foundation for the food preference categories. In particular, sports beverages, energy drinks, and smoothies were not included, although adolescents are by far the most frequent consumers, and the largest growing consumer group of energy and sports drinks (Heckman et al. 2010; British Soft Drink Association 2016). In addition, the measure of liking of fruit squash did not distinguish between sugar- or artificially-sweetened fruit squash. Future research studies should include these drink types.

Preference scores were based on self-report, which is subject to bias. It is possible that adolescents underreported liking for food or drinks typically considered as "unhealthy" probably due to social desirability bias. SSB consumption has previously been found to be underreported by up to 30-40% when validated against an objective blood biomarker (Davy et al. 2011), and this type of underreporting has been shown to increase with BMI (Bratteby et al. 1998). However, most participants in this study had a healthy BMI, and the questionnaire data were collected anonymously which would have helped to minimise bias. It is also possible that food and drink preferences are susceptible to short-term variation, influenced by recent food or drink consumption in the period preceding measurement (Griffioen-Roose, Hogenkamp, et al. 2012; Martin et al. 2011). However, the test-retest pilot study of this questionnaire indicated that beverage preference scores were reasonably reliable over a two-week period.

It was also not entirely possible to validate how closely self-reported preference actually reflects the intake of these adolescents. However, focusing on food and drink *preferences* rather than *intake* or *consumption frequency*, has several advantages. Arguably, *preferences* can be measured more accurately than actual intake, using a preference questionnaire that includes a comprehensive list of food and beverage types distributed to a large population sample; overcoming the high cost and inaccuracies of dietary *intake* assessments (Shim et al. 2014).

We only identified two twin studies that had investigated the aetiology of beverage preferences, and both examined coffee only. In addition, previous twin studies on food preferences have not investigated the genetic and environmental influences on food preferences in an adolescent sample before. Further research is therefore needed to replicate these findings, and to establish if the aetiology of food and drink preferences varies with developmental stage (e.g. toddlerhood, early childhood or adulthood). This is especially warranted given that energy consumed from drinks now accounts for a substantial proportion of energy intake in the modern diet, with roughly 20% of people's daily calories coming from beverages (Nielsen & Popkin 2004; Mesirow & Welsh 2015).

Rates of underweight (10%) were higher in this subsample of TEDS, and rates of overweight (13.4%) and obesity (4.5%) were low, relative to the UK population for this age group (in 2015 in the UK in this age group there were: underweight = 8%; overweight = 21%; obese = 16%) (NHS Digital 2015). The sample was predominantly white British so the results need replicating in more diverse samples. This indicates they may not be entirely representative in terms of dietary factors because they were considerably leaner. Nevertheless, participants were drawn from the TEDS study which has been shown to be reasonably representative in terms of important sociodemographic characteristics of the UK general population (Haworth et al. 2008). While these indicators do not include the primary variables under consideration, this observation suggests these findings were obtained from a sample representative of the

UK's young adult (18-19 years) population. Likewise, it was not possible to assess whether the food and drink preferences of this sample were comparable to the preferences of the UK general population. Consequently, caution is warranted before the results of the study are generalised to larger, more diverse populations. On the other hand, the large sample size and narrow age-range were strengths that allowed reliable estimates for food and drink preferences to be established for a specific developmental phase. A limitation of existing studies of adults is the very wide age range included in each analysis, with studies typically including individuals from early adulthood to older age, making it impossible to ascertain if influences are different for younger and older adults.

It was unknown whether the twins had already left home or were residing in a shared home at the time of data collection. The twin method relies on the assumption that MZs and DZs share their environments to a very similar extent, so if more MZs lived together than DZs (or had more frequent contact), the MZ similarity could be inflated, artificially inflating heritability. Although it was not possible to rule this out, previous studies have tested the equal environments assumption and found it to be valid in young and later adulthood (Conley et al. 2013).

5.5.6 Implications and future research

As this was the first study to investigate preference for non-alcoholic beverage types, these broad beverage preference categories will serve as a base to inform future studies which aim to investigate beverage consumption behaviours in more detail. For instance, it would be valuable to investigate the aetiology for the liking of water, which is the beverage type consumed most frequently in this age group (Özen et al. 2015).

These results suggest that food and drink preferences are a reasonable target for genome wide associations studies that seek to identify common genetic variants associated with variation in a range of food preferences. Further research is needed to characterise the biological pathways from genes to behaviour.

The findings from this study supports a growing area of research that has focused on understanding the environmental shapers of food and drink preferences. The knowledge that unique environmental factors are important determinants of food and drink preferences in adolescence is useful for identifying potentially modifiable risk factors to improve preferences for 'healthier' foods (and potentially reduce preferences for 'unhealthier' foods) for the population. Preferences are one of the most important drivers of actual intake and have been identified as useful predictors of nutrition-related disease risk (Duffy et al. 2009). Understanding the origins of *preferences* therefore provides useful insights into the factors that need to be targeted by public health programmes to modify consumption (e.g. decreasing intake of SSBs).

Current national and international obesity policies adopt a child-centric approach, focusing on regulating and restricting the obesogenic influences such as targeted advertisement of energy-dense food and drinks to children (World Health Organization 2010). Preventing excessive weight gain in childhood is a public health priority as a high BMI in childhood is a predictor of overweight or obesity in adulthood (Singh et al. 2008; Simmonds et al. 2015). However, adolescents' rates of obesity are rising more rapidly compared to the rest of the adult population (Allman-Farinelli et al. 2008), and maintenance of energy balance and healthier dietary intake in this age group has great potential for disease prevention (Votruba et al. 2014). While children are rightly considered vulnerable members of society (Calvert & Calvert 2017), it is short sighted to assume that once a child transitions into 'adolescence' they should be fully exposed to the unregulated commercial pressures of the permissive food and drink environment. Adolescence is an especially vulnerable developmental period during which individuals are particularly susceptible to social pressures (Freeman et al. 2015; Pechmann et al. 2005). The food and drink industry employ sophisticated strategies which capitalize on this vulnerability to influence adolescents' attitudes and consumption of their products (Bishop 2000). These findings suggest there might be further population health benefit to be gained if commercial strategies that target older adolescents are submitted to the same level of regulation as marketing strategies that target younger children (Nelson et al. 2008).

5.6 Conclusion

Food and drink preferences of older adolescents are influenced by both genetic and non-shared environmental influences. However, food and drink preferences in this sample were not influenced at all by aspects of the environment completely shared by twin pairs. This suggests that children's early shared family experiences relating to food and drink preferences may not have lasting effects, or that these effects dip through late adolescence but may reappear in later adulthood. In addition, this study emphasizes the importance of unique environmental factors in shaping preferences, highlighting the potential to modify preferences for unhealthy foods and beverages by national public health programs. Specifically, for SSBs, which have a strong evidence base for their detrimental health effects, and which can be relatively easily targeted by taxation strategies or levies. Policies targeting the wider environment are promising techniques to achieve substantial population level health gains (Briggs et al. 2017).

With the recent rise of national governments and jurisdictions worldwide enacting these fiscal measures, the results from this study suggest these initiatives might be particularly effective in adolescent populations. Efforts to improve adolescent food and drink preferences may, for that reason, be best targeted at the wider environment; strategies might include increasing the availability of, lowering the cost of, and promoting ‘healthier foods’ (Moorhouse, J., Kapetanaki, A. Wills 2015). This approach will require stronger government legislation and regulation of the food and drink environment (Hawkes et al. 2015; Hebden et al. 2015).

This study demonstrates that variation in food and drink preferences have a moderate genetic basis in older adolescents and that the remaining variation is influenced by the non-shared environment. The sizeable non-shared environmental influence on food and drink preferences highlights the potential for environmental modification. This introduces other questions on food and drink preferences, such as: “How do food and drink preferences relate to adiposity in older adolescence?”, and “How can food and drink preferences be modified in young adults?”. These questions have never been looked at before and are investigated in **Study 3** and **Study 4** in the following chapters.

Chapter 6.

6.1 Study 3a – Cross-sectional associations of food and drink preferences and BMI in older adolescents[I]

6.2 Introduction

The results from **Study 2** demonstrated that variation in adolescents' food and drink preferences is under the influence of both genetic- and unique environmental factors, with environmental factors accounting for at least half of the variation. These findings suggest older adolescents' food and drink preferences may be modifiable through interventions targeting aspects of the wider environment. To inform public health action in the context of the current obesity epidemic, it would be valuable to understand if specific food and drink preferences are related to adiposity. If preferences for specific food groups predict higher adiposity, these preferences could be targeted through appropriate interventions. On the other hand, it is also possible that food and drink preferences are not causally linked to adiposity in the general population, and that adiposity is mainly shaped by *how much* or *why* we eat (for example, eating for reasons other than hunger), rather than the *type* of food and drinks we consume.

As identified in **Chapter 1**, previous research suggests a possible association between higher preference for sweet and fatty foods and greater adiposity. However, the high degree of heterogeneity in the types of food preferences assessed (ranging from individual foods items to diverse and inconsistent food groupings), as well as in the sample characteristics makes it very challenging to draw any firm conclusions. The literature exploring associations between food preferences and adiposity has tended to focus almost exclusively on liking for foods high in fat and sugar. Thus, little is known about how preferences for dairy foods, meat, fish or grains and other starch foods might relate to adiposity. This is an important oversight, as higher preference for some of these foods may confer either protection from, or risk of, excess weight.

Additionally, there is a dearth of studies exploring the relationships between drink preferences and adiposity. Beverage consumption patterns have changed considerably over recent years, with marked increases in sugar-sweetened beverage (SSB) intake

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[I] Data from this chapter was presented as a poster on the 20th of Sept. at the Association for the Study of Obesity UK Congress on Obesity 2016 in Nottingham, UK: Smith, A.D., *Fildes, A. Cooke, L. and Llewellyn C. - Adolescent drink preferences and weight.*

(Adair & Popkin 2005), and there is strong evidence implicating SSB consumption in the development of obesity and overweight (Malik et al. 2006; Hu & Malik 2010). Yet the relationship between preferences for SSBs, or other common drinks, and adiposity remains unclear.

In the literature review undertaken in **Chapter 1**, only one study was identified that investigated food and drink preferences and adiposity in an adolescent sample (Diehl 1999). In this study, 1233 school-age boys and girls were asked to rate their liking of 114 food items and 14 different beverages on a 5-point facial hedonic scale. The highest preference ratings were reported for items such as hamburgers, ice cream, fruit, and starches (e.g. chips and pasta), whilst lowest ratings were generally seen for cooked vegetables, liver, and canned seafood. Overall, none of these preferences were significantly linked to BMI z-scores. However, participants in this study were younger adolescents (aged 10-14 years) all of whom still lived at home (Diehl 1999).

The relationship between food and drink preferences and adiposity has not yet been addressed in older adolescents during the transition into adulthood, when individuals gain increasing independence and begin to leave the family home. During this developmental period, there is a noticeable shift in interpersonal support systems, and new behavioural patterns are learned during emerging adulthood (Nelson et al. 2008).

As was established in **Study 1**, food preference scores are inter-related in young adults. In keeping with previous literature, preference scores for individual food items can be grouped into internally reliable and logical categories. The few previous research studies that have investigated the relationship between single item measures of food preferences and adiposity have found unreliable results (Laureati et al. 2015; Diehl 1999; Fisher & Birch 1995; Czarnocińska et al. 2009). In contrast, using aggregate, multi-item food preference categories allows the complex, multi-dimensional nature of dietary preferences accurately to be taken into account (Newby & Tucker 2004). It also increases power to detect small effect sizes which is challenging when single item preferences measures are considered in nutritional epidemiology; opting for multi-item categories to analyse the structure of self-reported food preferences thus makes sense (Gardner et al. 1998).

Energy density of food is an important influence on dietary energy intake (Stelmach-Mardas et al. 2016). Typically foods high in water and fibre-content, such as fruit and vegetables, have lower energy density (Bechthold 2014). On the other hand, higher fat content of food greatly increases energy density. Fat is the most nutrient dense macronutrient (9 kcal/g) followed by carbohydrates (4 kcal/g) and proteins (4 kcal/g) (Ello-Martin et al. 2005) consumption of foods with a high energy density increases energy intake in contrast to foods with low energy density (e.g. fruits, vegetables) (Bell & Rolls 2001; Rolls et al. 1999; Bell et al. 1998).

Identifying specific food and drink preferences associated with higher levels of adiposity could inform interventions, enabling modifiable dietary preferences to be selectively targeted in this age group to limit the risk of long-term weight gain. Both the snacks and dairy food preference category were largely composed of energy dense items, resulting in a tentative hypothesis that greater liking of these foods may be associated with greater adiposity. In contrast, greater liking of fruits and vegetables may be linked to lower adiposity.

6.3 Study aims

This study aimed to establish for the first time if preferences for six food groups and seven drinks types are associated with adiposity in older adolescents. It was hypothesised that greater liking for calorie-dense foods (e.g. snacks, dairy) and drinks (SSBs, milk) would be associated with higher BMI scores. On the other hand, it was hypothesised that greater liking for foods lower in energy density (e.g. fruits, vegetables) and lower calorie drinks (e.g. NNSBs) would be associated with lower BMI scores.

6.4 Methods

6.4.1 Sample

Study participants were 18-19-year-old twins from TEDS. The sample, recruitment, and measures are described in detail in Chapter 3.

6.4.2 Measures

6.4.2.1 Sociodemographic data

Age, sex and ethnicity were recorded in the baseline questionnaire collected when the twins were an average of 18 months old. The composite SES variable was derived from five variables assessing various aspects of parental occupation and education, as well as maternal age at birth of the first child. Information on ethnicity was collected at birth and dichotomized into two groups, a ‘White’ and ‘Other‘ group.

6.4.2.2 Food and drink preferences

Food and drink preferences were assessed using the food and drink questionnaire described in **Study 1**. Liking for six food groups (vegetables, fruit, meat/fish, dairy, snacks, and starches) and seven non-alcoholic beverages (SSBs, Non-Nutritive Sweetened Beverages [NNSBs], fruit squash, orange juice, milk, tea, and coffee) were measured using a 5-point Likert scale. Response options were coded from 1 to 5, with a higher value indicative of higher liking for the food or drink item.

6.4.2.3 Anthropometric data

BMI was calculated from self-reported weight (in kg) and height (in m). Study subjects (n=15) with implausible BMI values (<16 kg/m^2) were excluded from the final analyses for this study. BMI was also categorised into deciles by dividing the eligible sample into ten evenly-sized groupings calculated from the observed range of eligible BMI values (16.06 – 59.81 kg/m^2). For example, participants in decile 1 represent the 10% of the sample with the lowest body composition scores. Means and standard deviations for each food and drink preference score were calculated for each decile. Examining dietary preference scores across the entire BMI spectrum allowed me to establish if the relationship between food and drink preferences and BMI is linear.

6.4.3 Statistical analyses

6.4.3.1 Data screening

The food and drink preferences and BMI data were checked to ensure the assumptions of the analyses were met (parametric tests and CSGLMs). Normalities of the food and drink preference scales and BMI were inspected by investigation of skew and kurtosis statistics. Given the large sample size, visual inspection of histograms and descriptive statistics (defined as an absolute skewness >2 or <-2 or an absolute kurtosis >3 or <-3) were used to define substantial non-normality (Kim 2013). Some of the food and drink preference scores were skewed; normalization of the distribution was attempted using a logarithmic transformation (Log 10), but this was not successful (Tabachnick et al. 2001). Histograms of the preferences scores for both the raw- and transformed data are shown in **Appendix E1 and E2**. However, inspection of QQ plots (quantiles of preferences scores plotted against quantiles of BMI) indicated that error terms were normally distributed, suggesting that normality of the data was still a reasonably good approximation.

Further inspection of the data was undertaken by dichotomizing food and drink preference scores into ‘high likers’ vs ‘low likers’, split by median preference values. All CSGLMs were re-run with the dichotomized food and drink preferences scores as the independent variable to investigate how much these may explain variation in BMI, respectively. Models were adjusted with the covariates described above. In order to test the assumption of linearity (one of the assumptions of CSGLMs) in the relationship between food and drink preferences and BMI, polynomial contrasts were run to test for non-linearity (and linearity) of mean food and drink preference scores across BMI deciles.

6.4.4 Associations between food and drink preferences and BMI as a continuous variable

Correlations were calculated between BMI as a continuous measure and preference scores for the six food groups and seven drink types, to assess the strength and direction of their relationships. Bivariate associations between normally distributed variables were assessed using Pearson's product-moment correlation coefficients (r). Spearman's rank correlation coefficient rho (ρ) was used for variables that were non-normally distributed. Results are described in full detail in **Study 1**.

6.4.4.1 Complex Samples General Linear Models (CSGLMs)

Complex Samples General Linear Models (CSGLMs) were used to establish the associations between BMI, and food group and drink preferences adjusting for the clustering of the twins within families; thereby enabling the full sample of twins to be included in analyses (Carlin et al. 2005). CSGLMs were run with BMI as the dependent variable and separate models were run for each food category and drink preference type as the independent variable. Initially models were run unadjusted, but subsequently were adjusted for age, sex, ethnicity and SES to control for potential confounding by these factors.

For each CSGLM models, the slope of the regression line is shown by the unstandardized β-values; the stronger the relationship between the predictor and the outcome variable, the higher the value of the β-coefficient (Field 2013). The proportion of the variance in the outcome variable that is explained by the model is indexed by the coefficient of determination, R^2 (Field 2013). The higher the R^2 value, the stronger the relationship between the model predictors and the outcome variable. As stated in Chapter 3, the p-value was set at <0.01 for all analyses to take account of the large sample and multiple testing. All analyses were undertaken using SPSS Version 22.0 (SPSS Inc., Chicago, IL, USA).

6.5 Results

6.5.1 Summary statistics

After exclusion of n=15 study participants with implausibly low BMI values (<16 kg/m^2), an eligible sub-sample of 2850, out of the 2865 twins analysed in Studies 1 and 2, remained. Overall the sample was slightly more female (59.6%), had a healthy mean BMI of 22.4 (SD=4.15), and consisted largely of participants of white ethnicity (94.7%).

Sample characteristics of the twins included in the analyses are summarized in **Table 6.1**. A more detailed description of the sample and measures is provided in **Chapter 3.**

Table 6.1 Demographic characteristics of the study sample (n=2850)

Characteristic	Sample	
Sex [n (%)]		
Male	1152	(40.4)
Female	1698	(59.6)
Age [mean (SD)]	19.12	(0.28)
BMI [mean (SD)]	22.41	(4.15)
SES[1] [mean (SD)]	0.344	(0.95)
Ethnicity [n (%)]		
White	2698	(94.7)
Other	152	(5.3)

[1] SES entered as a composite standardised variable for n=2690 (mean range: -2.23, 2.49)

6.5.1.1 Data screening

As discussed previously in **Study 1**, mean food and drink preferences showed expected patterns of lower liking for vegetables (mean=3.66; SD=0.74) and higher liking for Snacks (mean=4.40; SD=0.53). The most popular drink type was orange juice (mean=4.43; SD=1.02) whilst the least preferred drink was NNSBs (mean=3.64; SD=1.34). **Table 6.2** summarizes the descriptive statistics for the food and drink preference scores.

Table 6.2 Descriptive statistics for mean food and drink preference scores

	Mean	SD	Median	Skew	Kurtosis
Food preferences					
Vegetable	3.66	(0.74)	3.72	-0.51	0.08
Fruit	4.24	(0.76)	4.43	-1.45	2.54
Meat/Fish	3.97	(0.70)	4.08	-0.91	1.11
Dairy	3.70	(0.71)	3.78	-0.62	0.26
Snack	4.40	(0.53)	4.56	-1.47	3.74
Starches	3.92	(0.68)	4.00	-0.67	0.51
Drink preferences					
SSBs[1]	3.73	(1.37)	4.00	-0.85	-0.58
NNSBs[1]	3.64	(1.34)	4.00	-0.74	-0.67
Fruit Squash	4.23	(1.02)	5.00	-1.54	2.02
Orange Juice	4.43	(0.97)	5.00	-2.01	3.71
Milk	4.23	(0.95)	5.00	-1.24	1.11
Coffee	3.85	(1.29)	4.00	-1.01	-0.13
Tea	4.31	(1.08)	5.00	-1.73	2.28

[1]*Abbreviations: SSB=Sugar-sweetened beverage; NNSBs=Non-nutritive sweetened beverages*

Overall, all mean food and drink preference scores were negatively skewed, with scores focused in the higher ranges of the preference scale. Mean preference scores for the liking of vegetables, dairy, starches, and coffee were close to the normal distribution. Preference scores for fruit, meat/fish, snacks, fruit squash, orange juice, milk and tea had positive kurtosis (a more peaked distribution) and SSBs and NNSBs had negative kurtosis (flatter distributions).

6.5.2 Covariates associated with food and drink preferences

Associations between sociodemographic variables (age, sex, ethnicity and SES) with BMI and preferences for the six food groups and seven drinks were tested using CSGLMs, as shown in **Table 6.3**. Higher SES was significantly associated with a lower BMI (β=-0.433 ± 0.084; p<0.001). None of the other sociodemographic variables were significantly associated with BMI.

Table 6.3 Associations of sociodemographic variables with BMI

	BMI		
	β (SE)	p value	R^2
Age (years)	.530 (.274)	.053	.001
Sex	-.258 (.160)	.106	.001
Ethnicity	.366 (.358)	.307	<.001
SES[1] (composite scale)	-.433 (.084)	<.001	.010

[1] SES entered as a composite standardised variable for n=2690 (mean range: -2.23, 2.49)

6.5.3 Relationships between food and drink preferences and BMI

6.5.4 Bivariate correlations

Pearson's correlations between BMI and preferences for the six food groups and seven drink types are shown in **Table 6.4**. There was a small but significant negative correlation between fruit liking and BMI (r=-0.05; p=0.01), such that higher liking for fruit was associated with having a slightly lower BMI, but the effect size was small. No other food group preference scores were significantly correlated with BMI.

Greater liking for fruit squash was significantly positively associated with BMI such that adolescents with higher liking for fruit squash had a higher BMI, although the effect size was very small (r=0.05; p=0.006). BMI was also significantly and positively associated with greater liking for NNSBs such that adolescents with a higher liking of NNSBs also had a higher BMI, but again the effect size was small (r=0.09; p<0.001).

Table 6.4 Correlations between BMI and food and drink preferences

	BMI		
	r[1]	p value	n
Food preferences			
Vegetables	-0.02	.35	2827
Fruit	-0.05	<.01*	2825
Meat/Fish	-0.01	.82	2825
Dairy	0.04	.03	2825
Snacks	-0.03	.03	2827
Starches	-0.02	.40	2827
Drink preferences			
SSBs[2]	-0.02	.52	2803
NNSBs[2]	**0.09**	**<.01**	**2789**
Fruit squash	**0.05**	**.01**	**2809**
Orange Juice	0.03	.08	2811
Milk	0.02	.23	2669
Coffee	0.01	.65	1879
Tea	0.01	.59	2382

Significant findings are bolded.
[1] Pearson's correlation coefficients were used for normally distributed mean scores, except for 'orange juice' where Spearman's rho was used.
[2] *Abbreviations: SSBs=Sugar-sweetened beverages; NNSBs=Non-nutritive sweetened beverages*

6.5.5 Complex samples general linear models

6.5.5.1 Food preferences

Table 6.5 shows the unstandardized coefficients from the unadjusted and adjusted CSGLMs for the associations between food and drink preferences and BMI, which control for clustering of twins in families. None of the six food preference categories were significantly associated with BMI in the unadjusted CSGLMs. Following adjustment for age, sex, SES and ethnicity, higher liking for dairy was positively and significantly associated with BMI (β =.299 ± .112, p=.008); each one unit increase in liking for dairy (e.g. "like a little" to "like a lot") was associated with an increase in BMI of 0.30 kg/m^2. The difference between those scoring highest and lowest for dairy liking (e.g. "dislike a lot" and "like a lot") was 1.20 BMI units.

Because food preference scores were negatively skewed they were also entered as dichotomized variables into the fully-adjusted CSGLMs, split by the median preference score into 'low' and 'high' likers (**Appendix E6**). No significant associations were found between being a high or low liker with BMI for any of the six food categories.

6.5.5.2 Drink preferences

In keeping with the Pearson's correlation coefficients, there were positive significant associations between BMI and liking of NNSBs (β =.285 ± .066, p<.001) and fruit squash (β =.195 ± .076, p=.010) in the unadjusted CSGLMs (**Table 7**). A one-unit increase in liking for NNSBs (e.g. "like a little" to "like a lot") was associated with an increase of 0.29 BMI units, and a one-unit increase in liking for fruit squash was associated with an increase of 0.20 BMI units. However, only the association between higher liking of NNSBs and BMI (β = .277 ± .070, p<.001) remained significant after adjustment for age, sex, SES and ethnicity, and the effect size was virtually unchanged: a one-unit increase in liking for NNSBs was associated with an increase of 0.28 BMI units. This equated to a difference of 1.12 BMI units between participants scoring minimum and maximum for NNSB liking (i.e. "dislike a lot" and "like a lot").

In line with these findings, when drink preferences were entered as dichotomous variables 'low' liking for NNSBs was significantly negatively associated with BMI, such that in the fully adjusted CSGLM the BMIs of 'low' and 'high' likers differed by 0.77 kg/m^2. There were no other significant associations between dichotomized drink preferences and BMI (**Appendix E3**).

Table 6.5 Associations of food and drink preference scores with BMI (continuous)

	BMI			BMI[1]		
	β(SE)	p value	R^2	β(SE)	p value	R^2
Food preferences						
Vegetables	-.099 (0.120)	.409	<.001	-.007 (.124)	.957	.013
Fruit	-.258 (0.123)	.036	.002	-.227 (.126)	.073	.015
Meat/Fish	-.025 (0.116)	.830	<.001	.085 (.123)	.487	.013
Dairy	.241 (0.111)	.030	.002	**.299 (.112)**	**.008**	**.016**
Snacks	-.310 (0.161)	.054	.002	-.321 (.162)	.047	.015
Starches	-.097 (0.130)	.454	<.001	-.054 (.134)	.688	.013
Drink preferences						
SSBs[1]	-.052 (.063)	.410	<.001	-.045 (.066)	.496	.013
NNSBs[1]	**.285 (.066)**	**<.001**	**.008**	**.277 (.070)**	**<.001**	**.020**
Fruit squash	**.195 (.076)**	**.010**	**.002**	.140 (.077)	.071	.014
Orange juice	.012 (.100)	.907	<.001	-.028 (.102)	.786	.013
Milk	.103 (.086)	.231	.001	.101 (.092)	.271	.014
Coffee	.033 (.079)	.674	<.001	.068 (.075)	.363	.017
Tea	.043 (.085)	.618	<.001	.044 (.086)	.611	.013

Significant findings are bolded.
[1] Model was adjusted for sex, age at questionnaire completion (years), socioeconomic status (composite scale) and ethnicity
[2] *Abbreviations: SSBs=Sugar-sweetened beverage; NNSBs= Non-nutritive sweetened beverages*

6.5.6 Testing for non-linear effects

There were no clear patterns of significant non-linear trends looking across BMI deciles for any of the food and drink preference scales, indicating that null findings in the CSGLMs were not the result of non-linear relationships between food and drink preferences and BMI. There were quadratic effects for starches and meat/fish but pairwise mean differences across groups, finding only a difference between deciles 7 and 10 for meat/fish, and between deciles 2 and deciles 4 for starches. For fruit squash and NNSBs there were significant linear as well as quadratic effects. Again, pairwise comparisons did not indicate any clear quadratic pattern, so it is appropriate to focus on the findings from CSGLMs. Summary statistics (means and SDs) for the food and drink preference scores across BMI deciles are shown in **Appendix E4**. Bar charts of mean food and drink preferences scores by BMI deciles are shown in **Appendix E5 and E6**, respectively.

6.6 Discussion

6.6.1 Summary of findings

This study is the first to explore the relationship between preferences for a broad range of food and drink categories and adiposity in older adolescents. Contrary to the hypothesis, higher liking for typically energy-dense food groups or drinks was not positively associated with higher BMI. One interesting finding was that that a higher preference for dairy foods was associated with a higher BMI. This partly supports the hypothesis that higher liking for fatty foods (higher in energy density) is associated with higher BMI No such trends were observed for snacks (examples of energy-dense sweet and savoury foods), or for SSBs, orange juice and milk (examples of energy-dense drinks). In addition, there was no evidence that greater preferences for foods and drinks lower in energy density had any proactive effect on BMI. Higher liking for fruit and vegetables (examples of nutrient-dense, less energy-dense foods) were not inversely associated with BMI, whereas higher liking for NNSBs (a lower energy dense beverage) was associated with a *higher* BMI.

6.6.2 Findings on the association between food preferences and BMI

Notably, greater liking for dairy foods was positively associated with BMI. However, the relationship between dairy intake and adiposity is far from fully understood, with studies reporting both negative and positive associations between dairy intake and adiposity (Kratz et al. 2013; Huth & Park 2012; Mirmiran et al. 2005; Wiley 2010). This may be partly due to the diversity in dairy foods commonly available (Astrup 2014): full fat or low fat, sweetened or unsweetened, in liquid or solid form, or even fermented.

Preference and intake for each of these foods may differ due to variations in satiation effects, palatability and texture. Observational data pooled from three US prospective cohorts found that higher consumption of yogurt, and to a lesser extent the intake of cheese, was significantly associated with decreases in adiposity over a four-year period (n=120 877; mean baseline age range: 37.5 – 52.2 years) (Mozaffarian et al. 2011). Milk consumption (skimmed milk and whole milk) was not significantly associated with weight change in the same study. Short-term changes in bodyweight and dairy consumption have been quantified in two meta-analyses of randomized controlled trials (Chen et al. 2012; Abargouei et al. 2012). In the first meta-analysis, increasing dairy intake to the recommended level in the absence of caloric restriction resulted in small weight loss effects, and trials which included caloric restriction as part of their protocol were on average more effective in achieving significant weight loss. Consistent findings were reported in the second meta-analysis that pooled data from 22 randomized controlled trials. Overall the results from this meta-analysis suggest that increased dairy intake, in the short-term only, or coupled with caloric restriction, is effective in facilitating weight loss (Chen et al. 2012). However, these trials had once again only focused on intake rather than preference for dairy items, and were mainly undertaken in participants actively attempting to lose weight.

In **Chapter 1**, three studies were identified which used food and drink preference questionnaires that included various dairy items, but none included dairy as an explicit food category of interest. Two of these studies were in children. Hill et al collected preference ratings for ice cream and yoghurt in a sample of 6-9 year olds (n=366). These preference rating were incorporated into a mean 'fatty/sugary food' group score along with other items (e.g. plain biscuits, crisps, and cakes) (Hill et al. 2009). In a sample of slightly older children (10-14 years; n=1233), single item food and drink preferences were measured and correlated with self-reported BMI z-scores. The extensive questionnaire used in this study featured ice cream, milk, yoghurt, butter cream cheese, hard cheese, and milk. Neither study found significant associations between preferences for dairy foods and adiposity. In comparison, a third study undertaken in adult males (n=422) did find significant positive associations between higher liking for 'fatty foods' (which included preference ratings for cheese, butter, milk, sour cream, mayonnaise, whole milk and whipped cream) and BMI or waist circumference (Duffy et al. 2007). Notably, most of the dairy items included in the three studies that measured dairy preference, as opposed to intake, were predominantly processed and high in fat or sugar. This may limit meaningful conclusions as these items could be considered more similar to 'snack' foods than the food items grouped in the dairy food category of the food preference questionnaire used in the current study.

In addition, the inclusion of non-dairy items (e.g. eggs or mayonnaise) in the 'dairy' food category preference scale of the present study may have distorted the observed relationship between liking for dairy foods and BMI.

Previous research has suggested that one mechanism by which gustatory preferences may be influencing adiposity is via a higher hedonic appreciation for fatty and creamy tastes in foods. Foods high in fat are smooth and creamy, which make them highly palatable and encourages overconsumption (Drewnowski & Greenwood 1983). The dairy category used for this study included items such as mayonnaise, butter, butter-like spread, and cream, which have exactly these sensory attributes. The finding that the liking of dairy is positively associated with BMI is compatible with the conclusion of a recent systematic review (Cox et al. 2016) of the relationship between liking or preference for the taste of fat in foods and various measures of adiposity in adults and children. Eight of out 13 studies found positive associations, and among these were two studies that found a significant positive association between liking for the taste of fat and weight status (Drewnowski et al. 1985; Duffy et al. 2009). The other six significant studies also found positive associations between higher preference ratings for fat or fat taste, with measures of adiposity. (Duffy et al. 2007; Mela & Sacchetti 1991; Ricketts 1997; Matsushita et al. 2009; Fisher & Birch 1995; Lanfer et al. 2012). Four studies found no association, which may be due to the choice of fat preference measures used. All four of the studies which reported no association between fat preference and weight relied on forced-choice comparisons of higher-fat versus low-fat versions of foods (e.g. cheese, milk, cake icing, yogurt etc.), and associated difference in preference rankings to adiposity status (Drewnowski et al. 1991; Stewart et al. 2010; Alexy et al. 2011) or adiposity (Salbe et al. 2004). However, in a previous experimental study on toddlers (18-37 months; n=74) it was found that sensory detection of fat in food may not be well-detected by humans (Bouhlal et al. 2011). In this study, researchers served ad-libitum lunches to toddlers in their nurseries which had been manipulated to contain varying concentrations of fat and salt. Food intake was unaffected by the manipulation of fat content, while stronger salt concentrations resulted in a noticeable increase of food intake. There was only one study that found a negative correlation between the liking of high fat foods and weight status (Cox 1998). The conclusion of the review is also consistent with a previous study which demonstrated that children at higher risk for obesity as indexed by parental adiposity status (n=200; aged 4-5 years) show higher liking for the taste of fatty foods compared to lower risk children. The children were asked to taste and rank six different foods with varying fat contents (Wardle, Guthrie, et al. 2001). Average ranked preference ratings for higher fat foods (i.e. chocolate, cheese, and butter biscuits) were calculated and higher liking was positively associated with higher BMI.

All in all, these findings do provide evidence of a relationship between preference for fat and adiposity status. However, it is important to consider that these observations may in fact be the result of reverse causation, such that liking for fat-rich foods is the consequence of body adiposity status and not the cause (Mattes 1993; Drewnowski et al. 1985), and this also may in-part explain the observed association between higher liking of dairy foods with BMI in the present study.

On the other hand, higher vegetable liking was not associated with lower BMI, despite previous evidence in support of the weight protective benefits of vegetable consumption (Boeing et al. 2012). Most evidence linking vegetable consumption and adiposity has been collected from prospective studies. These findings include inverse associations between vegetable intake and weight gain over 12 years among women, and over 4 years among men and women in the US (He et al. 2004; Mozaffarian et al. 2011). Other studies have reported a significant decrease in the relative risk for weight gain in Spanish adults (>3.14 kg over 10 years), or in Japanese adults (>3kg over one year), in those individuals that reported the highest intake of vegetables (Sawada et al. 2015; Vioque et al. 2008).

This finding was partially consistent with two other studies that have previously investigated the relationship between vegetable *preference* (not *intake*) and adiposity. The first study of 7-9 year old school-children (n=366), measured liking for 'vegetables' as a sum score of 24 vegetable preference ratings from a food preference questionnaire. Mean preference ratings for this category were not associated with BMI z-scores (Hill et al. 2009). The second study was also undertaken in a paediatric sample (n=528; 6-9 y). However, in this study preference scores for four vegetables (fennel, radish, broccoli and carrot) had been measured using a laboratory-based taste test with preference ratings recorded on a 7-point Likert scale; none of the vegetable item preference scores were significantly associated with adiposity (Laureati et al. 2015). Vegetable consumption has been mainly implicated as weight protective due to the low energy density and high-fibre content of these items. However most of the evidence of the relationship between body weight and vegetable consumption from intervention studies does not focus on the effect of vegetable intake only (Rolls et al. 2004). Commonly, recommended increases in vegetable intake are accompanied by recommendations to increase fruit intake too, making it difficult to isolate the effect of vegetable consumption on weight management (Ledoux et al. 2011).

It is also important to emphasize that higher liking for fruits and vegetables can only protect against overweight if higher preference translates into higher intake of these foods, which in turn displaces more energy-dense foods from the diet (Mytton et al. 2014). Regardless, there has been one previous study in 4-5-year old children that compared food preferences of children at high risk of obesity (indexed by having two obese parents) with food preferences of children at a lower risk of obesity (with two lean parents). Children from the lean families had significantly higher preference scores for vegetables. The authors concluded that an inherited dislike of vegetables (along with a higher preference for fat in food, and more time spent engaging in sedentary behaviours) may play an integral role in the pathway whereby genetic risk of obesity is transmitted to the next generation (Wardle, Guthrie, et al. 2001). It is also possible that the absence of an inverse association between higher liking for vegetables with adiposity in the present sample, is because people who enjoy lots of different foods (including vegetables) often have higher BMIs (J. Wardle et al. 2001; Hunot et al. 2016). Interestingly, a recent study in British (n=1044) and Australian (n=167) children found that this preference may develop at a young age, as vegetable (and fruit) preference was positively associated with enjoyment of food in a sample of 16-month (UK) and 3-4 year olds (Australia) (Fildes et al. 2015).

Liking for fruit was not related to BMI. It was hypothesized that higher liking for fruit may protect against excess weight. This makes intuitive sense, given that fruits are relatively high in water and fibre content, and relatively low in dietary energy. The initial hypothesis was also supported by findings from an extensive review of the literature, where most of the 16 identified studies reported associations between higher fruit intake and lower BMI scores. Overall, 2/3 randomized controlled trials in overweight or obese individuals found that increases in fruit intake resulted in significant weight reduction compared to control groups (which were not supplied with fresh fruit or were instructed to follow a low calorie, low fruit diet). Longitudinal findings (following study subjects for 2-8y) from 5/8 studies linked higher fruit consumption to significantly lower relative risks for weight gain (n=3) and decreases in body weight (n=1), and one study observed that decreases in fruit consumption were associated with weight increases (n=1) in an overweight population. In 4/5 of the cross-sectional studies that examined the association between mean fruit servings consumed per day and body weight, significant inverse associations were reported. However this was the case for women only in two of the studies, suggesting there may be sex differences (Alinia et al. 2009). There are some limitations from these studies that need to be considered. For example, the intervention studies were undertaken in overweight individuals who expressed a desire to reduce their weight, meaning that the association of higher fruit intake with weight loss cannot necessarily be extrapolated to normal weight individuals. Fruit intake in the observational studies was self-reported, which may have overestimated actual fruit consumption due to social desirability bias. Furthermore, measures of fruit consumption in the cross-sectional and prospective studies rarely considered the preparation form of the reported fruit intake (e.g. raw, dried or juice), an important aspect of fruit intake that may have affected the observed relationship with adiposity.

The null association between liking for fruit and BMI in the current analysis is in line with previous research in two studies of children. Mean preference rating for 10 common fruits did not significantly predict BMI-SD scores in a sample of 7-9 y old children (n=366) in the UK (Hill et al. 2009). Similarly, liking for four raw fruit samples rated on a 7-point facial hedonic scale in Italian school children (6-9 y; n=528) were unrelated to BMI z-scores (Laureati et al. 2014). It is possible that the relationship between liking and intake of fruit is particularly weak. It is also important to consider that fruits are intrinsically sweet tasting foods. It is thus possible that greater preference for fruit may correlate with higher liking for sweet foods more generally, which would not be protective of weight gain.

Food items included on the snack preference scale were all highly palatable and energy-dense, e.g. chips, chocolate, biscuits, and cake. The result that higher liking of snack foods was not associated with higher BMI was therefore somewhat unexpected and not in line with the initial hypothesis. However, the finding is consistent with previous research on the relationship between the liking of snack foods and adiposity. In a study of primary school-aged children in the UK, no association between preference ratings for fatty and sweet food with BMI was found (Hill et al. 2009). Preference ratings for 24 high-sugar items by Canadian adults (n=151) also did not show a significant association with BMI scores (Davis et al. 2007). Surprisingly, in a sample 10-14 year olds (n=696), liking for snack foods such as sweet pancakes, cake, cookies, chocolate, chocolate bars and boiled sweets, was negatively associated with z-BMI scores, although in boys only (Diehl 1999). In the current study the null association between liking for snack food and BMI may have been partly attributable to the high mean score for snack food liking (4.4 out of a maximum score of 5), resulting in ceiling effects. People generally show high preferences for sweet and energy dense foods, making it harder to capture individual differences in this trait. It is also possible that older adolescents restrict their consumption of snack foods to manage their weight and their reported preferences reflect this. Particularly with snack foods, liking and intake are probably less related than with other food. Relating to this, social desirability bias may explain the observed association such that overweight/obese adolescents feel self-conscious about their weight and so report lower liking for unhealthy foods.

Preference scores for starch foods and the meat/fish food category were also not associated with adiposity in this study. It is challenging to compare these findings with previous research because no existing studies have assessed the relationship between preferences for these particular food groups, and BMI. A small study by Duffy and colleagues included liking for starch foods (e.g. oatmeal, wheat bread and potato) in a mean preference score for a 'fibre-rich' food preference group which additionally encompassed preference ratings for fruits and vegetables (Duffy et al. 2009). Greater preference for fibre-rich foods was positively associated with BMI in adults (n=88). However, the varied mix of items included in this food group limits any meaningful comparisons.

Likewise, the study of meat/fish as a distinct food preference category in relation to adiposity has only been investigated in one previous study. In this study of Japanese adults (n=892), liking for fat-rich meat was significantly associated with higher waist-to-hip ratio, abdominal skinfold thickness, and subscapular skin thickness in males, and with subscapular skin thickness was significant in females (Nakamura et al. 2001). Another study provided evidence of a significant positive correlation between higher liking for meat-based 'fatty foods' (beefsteak, sausage, and fried chicken) and BMI in adults (Duffy 2009). Again, it is not meaningful to compare findings from this study with the results seen for the meat/fish group in the current study because of the great disparity in food items making up the 'fatty foods' scale. In contrast to the high fat meat items included in the 'fatty food' group, items included in the meat/fish preference category included lean proteins such as chicken, ham, and white fish. This heterogeneity in measurement of food preferences means that both factors are tapping into different aspects of food liking, and therefore measuring different relationships with adiposity.

A plausible explanation for null association between liking of starch foods or meat/fish with adiposity may also be due to fact that both food groups are considered 'core foods'. This means that these are foods not typically eaten for hedonic reasons. It is generally established that palatability and 'wanting' for food are a driving force of overconsumption and have been implicated in the aetiology of obesity (Mela 2006; Blundell & Finlayson 2004), yet items on the starch and meat/fish scales would not typically be considered as highly palatable and thus don't show a strong relationship to weight gain in a relatively young population sample.

6.6.3 Findings on the association between drink preferences and BMI

There was only one consistently significant relationship between drink preference and BMI. In *unadjusted* models, higher preferences for both NNSBs and fruit squash were associated with higher BMI. However, the association between fruit squash and BMI disappeared after adjustment for SES, age, sex and ethnicity.

A positive association between NNSB preference and BMI remained significant after adjustment for SES, age, sex and ethnicity, such that higher liking for NNSBs was associated with higher BMI. For NNSBs, maximum versus minimum liking scores were associated with a BMI difference of more than 1 unit (1.12 kg/m^2). NNSBs are beverages of low energy-density, so this finding was unexpected. However, it is possible that this association is the result of adolescents who are engaging in weight management, choosing low-caloric drink options over SSBs (Pereira 2014). For example, an overweight adolescent who wants to lose weight may switch their usual SSB (e.g. Coca Cola) for a diet version (e.g. Diet Cola) and this process may increase their liking for NNSBs over time with increasing exposure.

The relationship between NNSB preference and BMI is probably complex, given that the relationship between NNSB *intake* and BMI is unclear even in rigorous epidemiological studies. Pereira (2014) recently reviewed the evidence from prospective and randomised trials on NNSB intake and body weight in adults and children. No consistent patterns across the 11 prospective studies could be identified (Pereira 2014). An inverse relationship between NNSB consumption and BMI was seen in 5/11 studies. Three longitudinal studies found no significant association, and only one study found a statistically significant inverse relationship between NNSB intake and adiposity. Findings from intervention studies are equally inconsistent. Overall, 3 randomized trials were identified which independently estimated shorter-term effect of NNSB intake on adiposity. Of these three trials, two found that the participants consuming SSBs gained adiposity, compared to the participants in intervention group that had been instructed to substitute SSBs with NNSBs over the course of 3 to 10 weeks, respectively (Tordoff & Alleva 1990; Raben et al. 2002). The third trial however found no differences in body weight change across four intervention groups, over a period of 6 months. Interestingly, only SSBs significantly increased hepatic fat accumulation compared to the other intervention groups which each had been separately randomised to drink 1 L of NNSBs, milk or water per day (Maersk et al. 2012).

It is also possible that *increased liking* for NNSBs plays a causal role in excessive weight gain. Several mechanisms have been proposed to contribute to this phenomenon. For example, higher liking for NNSBs may increase an individual's general sweetness threshold, and therefore increase intake of sweetened foods and drinks (Roberts 2015). There is also considerable interest in the potential for NNSBs, to cause alterations in metabolic health that may predispose to weight gain (Fernstrom 2015; Suez et al. 2015). Consumption of artificial sweeteners has been shown to upregulate the production of digestive hormones and thereby enhance the absorption of glucose (Brown et al. 2009), while simultaneously activating insulin signalling which stimulates hunger and promotes energy intake (Jang et al. 2007). More recently, artificial sweetener consumption has been implicated as a key dietary component contributing to the disruption of phylogenetic diversity of the gut microbiome (Suez et al. 2014). This imbalance in host-microbe interactions increased dietary energy extraction and may eventually increase the risk of weight gain (Payne et al. 2012).

Further evidence suggests that artificial sweeteners in NNSBs may stimulate hedonic brain responses differently than nutritive sweeteners (Smeets et al. 2011). It has been shown that nutritive sweeteners elicit strong activation of certain hedonic brain regions but recent functional magnetic resonance imaging studies have discovered that artificial sweeteners only result in incomplete stimulation of these brain regions. This incomplete activation may drive behaviours of overcompensation. Additionally, psychological overcompensation (also known as the 'self-licensing effect) because of NNSB consumption has also been proposed as a plausible explanation for the observed inverse association between NNSB intake and BMI. Individuals may reward themselves with extra food if they feel they are making the healthier (i.e. lower calorie) choice when they select a diet version of a soft drink over the full sugar version (Lavin et al. 1997).

Evidently there are substantial methodological limitations of both observational and experimental studies on this topic, primarily in the accurate measurement of dietary intake and in the design of analytical strategies to deal with the many biases inherent in the study of dietary behaviours and adiposity in human populations. RCTs are probably the best way to tease out cause and effect in NNSBs and adiposity gain/loss. But RCTs can't really be used to explore how naturally occurring variation in preferences for NNSBs relate to adiposity. This needs to be addressed by prospective studies that measure liking of NNSBs *before* individuals develop overweight to understand if liking of NNSBs is the cause or result of higher levels of adiposity.

Greater liking for SSBs was not associated with BMI in this study. This finding was somewhat surprising, given the many systematic reviews and meta-analyses (using rigorous evidence from longitudinal studies and randomised controlled trials) that have established positive associations between higher SSB consumption and excess adiposity (Te Morenga et al. 2013; Malik et al. 2006; Crawford et al. 2008; Gibson 2008; Wolff & Dansinger 2008; Woodward-Lopez et al. 2011; Olsen & Heitmann 2009; Drewnowski & Bellisle 2007). This discrepancy could be attributed to the fact that higher liking for SSBs does not translate to a higher intake of SSBs. Adolescence is a stage where body image is a prominent concern (Ata et al. 2007; O'Dea 2013). At the same time, SSBs have been widely reported as contributors to weight gain and obesity in the media. To conform to general western beauty standards, which promote a lean BMI as desirable, adolescents may avoid sugary drinks, regardless of preference.

The relationships between preferences for different drinks and adiposity remain largely unexplored. Only 3 studies were identified in the literature review (**Chapter 1**) that assessed liking of different beverage types, and of these, only one reported separate associations between beverage preferences and BMI. In this large study of German school children (n=1233), preferences for 14 different beverage types (e.g. apple juice, orange juice, cola, lemonade, hot chocolate, fizzy water, fruit tea, herbal tea, milk coffee, vegetable juice) were collected on a 5-point facial hedonic scale. Beverage preference scores were not significantly associated with adiposity for any of the 14 drinks. This study however had some important limitations. Primarily the young age of the sample means that exposure and consumption of different drink types - important influences on the development on preference formation - will have been restricted by parental control. Secondly, this study was undertaken in 1999; a time when beverage consumption patterns were very different to today. Future research studies would benefit from prospectively assessing preferences for a wider variety of beverage types (e.g. energy drinks, fruit smoothies), to gain a better understanding of the association between liking for a broad range of drink types and adiposity.

6.6.4 Food and drink preferences as important factors that shape BMI in adolescents

For energy-dense foods and drinks, only higher liking for dairy foods was found to positively predict BMI. The strongest relationship was seen between higher liking for NNSBs with higher BMI. The effect sizes of the associations were, however, small; but this is unsurprising given the many complex influences known to shape actual food choice (a closer determinant of BMI than preference), let alone adiposity (Köster 2009; Story et al. 2008).

From the age of 3 years until the onset of adolescence, food preferences are relatively stable (Nicklaus et al. 2004). Adolescence however has been identified as a key period of change in influences on food preferences because of growing autonomy over dietary choices. At the same time, social eating occasions with friends outside of the home become more frequent and other external factors such as ideologies about health and body weight mature (Nu et al. 1996). This means that adolescents' food choices may be informed by health or weight awareness rather than simply preferences (Visschers & Siegrist 2010). It is possible that food and drink preferences measured at 18-19 years of age may not fully reflect actual food choice, and therefore show limited associations with adiposity.

6.6.5 Strengths and limitations

This was a large study that used a wide-ranging food and drink preferences questionnaire to explore relationships with adiposity in older adolescents during an important developmental transition – from childhood to adulthood. However, there were several limitations. Firstly, and most crucially, this was a cross-sectional study, which means it is not possible to establish the direction of the relationship between food and drink preferences and adiposity. While it is plausible that higher liking for dairy confers a risk of excess weight, it is also possible that adiposity itself plays a causal role in food preferences. The same is true of the positive association seen between BMI and NNSBs. It is possible that individuals with BMIs in the overweight or obese range have adopted a calorie-controlled diet, typically a diet richer in fruit and vegetables, lean meat and fish, and low in processed and energy-dense foods (e.g. the snacks or SSB category), which in turn may have modified preferences.

The present study was not able to account for other important factors that may mediate direct effects of food and drink preferences on adiposity in adolescents. No measures of appetite, physical activity, financial situation, nutrition knowledge, dieting behaviours, or living situation were included in the questionnaire. These demographic, lifestyle and socioeconomic factors impact a person's motivation and ability to consume food and drinks that align with their preferences. Especially for fruit and vegetable intake, nutrition knowledge mediates actual intake (Wardle et al. 2000). Appetitive traits such as enjoyment of food have been shown to be associated with both higher adiposity (Sleddens et al. 2008; Wardle, Guthrie, et al. 2001), and higher preference for fruits and vegetables in children (Fildes et al. 2015). Individual variation in appetite is also likely to mediate the effect of food and drink preferences on adiposity. An obvious confounder affecting the relationship between food and drink preferences with BMI is total energy intake, which may distort the observed associations. It is always impossible to completely control for potential confounders, even if they are measured – because we can never measure anything perfectly. But discordant MZ twins allow us to study the association unconfounded by all unmeasured environmental factors shared by twin pairs, as well as genetic confounding.

Nevertheless, this study considered the relationship of a comprehensive range of food and drink preferences with BMI in a large population-representative cohort. Most previous studies were in small groups (majority n<600) and consisted of individuals across diverse age ranges (e.g. including children and adults in the same analyses). Importantly, dietary preferences were grouped so that associations between each food and drink preference category and BMI were independently investigated. Additionally, the food groups used in the analyses have a robust factor structure and are internally reliable (as shown in **Study 1**).

Underweight in this age group has been associated with eating disorders (Flament et al. 2015). While participants classed as 'severely underweight' (BMI <16 kg/m^2), were excluded from the analyses (n=15; BMI values 9.51 – 15.98 kg/m^2), the inclusion of underweight participants (BMI 16 – 18 kg/m^2), with potentially different patterns of food preferences, may have distorted some of the associations between food and drink preferences with adiposity.

The participants were predominantly white and lean (the mean BMI of the sample was 22.4 kg/m^2) limiting the generalisability of these results to the general population. Twin growth trajectories differ from those of singletons (van Dommelen et al. 2008). These differences decline by the age of 2-3 y but never fully disappear. For this reason, it would be important to replicate the result from this study in a larger and more population-representative sample.

Food and drink preference scores and weight and height were self-reported, which may have resulted in social desirability bias – adolescents consciously or subconsciously modifying their preference ratings for items that they perceived as 'unhealthy'. This may have been more pronounced at the higher end of the weight spectrum; for example, overweight or obese individuals may have been more likely than healthy weight individuals to over-report their liking for 'healthier' foods, or under-report their liking for ‘unhealthy’ snack foods. However, the food and drink questionnaire was administered online and data were anonymised, minimising the likelihood of such bias.

6.7 Conclusions and implications

This was the first study to characterise the cross-sectional relationships between a comprehensive number of food groups and drinks, and BMI in older adolescents. The findings suggest that in older adolescents, higher liking for dairy foods and NNSBs is associated with higher BMI. However, the effects sizes were small. Relationships between preferences for certain foods and drinks with BMI are complex, and the directions of the relationships cannot be determined using cross-sectional data. In addition, adolescents are still relatively young and healthy, and they have only recently gained full control over their dietary intakes.

These results may be useful for those who are already developing intervention studies to modify food and drink preference in this age group as they suggest that the complicated relationship between beverage preferences (especially SSBs and NNSBs) warrants further investigation with respect to adiposity. Importantly, it may be possible to collect these data again in this sample in a few years to test the direction of the relationship between BMI and food and drink preferences, and to establish if they change over time.

In conclusion, the findings from this study suggest that there are only limited relationships between preferences and adiposity in this age group and preferences do not seem to be a key driver of BMI.

Study 3b – Cross-sectional associations of food and drink preferences and BMI using a same-sex discordant twin design [ll]

6.8 Introduction

The results from study 3a highlighted that the relationships between food and drink preferences with BMI in an older adolescent sample are complex. Some associations between certain food and drink preferences with BMI were established at the individual level but the causal role of these preferences in adiposity variation remains unclear because the temporality of the observed exposure-outcome findings cannot be determined. A more sophisticated study design is required to overcome some of the limitations of the cross-sectional nature of the data. Taking full advantage of the genetically-sensitive nature of the TEDS data, the findings from Study 3a were further explored using a methodology known as the 'discordant MZ twin design'.

Body weight is highly heritable, with previous studies estimating genetic factors to account for around 80-90% of the variance in BMI at age 19 (Dubois et al. 2012). The remaining variation is shaped by factors from the non-shared environment. Discordant twin designs offer a powerful method for studying the relationship between a 'risk factor' (e.g. certain food and drink preferences) and an outcome (e.g. BMI), controlling for potential confounding by unmeasured differences between the aspects of the environments of unrelated individuals which might be responsible for differences in both food and drink preferences *and* BMI (e.g. SES, food availability in the home or school, parental food policies). Twins raised in the same household share many aspects of their environment, such as experiencing similar developmental events (e.g. divorce, moving to a new house, changing schools), as well as other influences of the family, home and school, and they are exactly the same age. Identical twins also share 100% of their genes (and are the same sex) allowing for potential genetic confounding in the relationship between two traits to be completely accounted for (D'Onofrio et al. 2003). Genetic confounding is called pleiotropy, and leads to two traits being phenotypically associated because they are caused by the same genes influencing

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[ll] Data from this chapter was presented on the 7th of September as an oral presentation at the Association for the Study of Obesity (ASO UK Congress on Obesity 2017 in Pontypridd, Wales:
Smith, A.D., Fildes, A. Cooke, L. and Llewellyn C. - The food and drink preferences of twins discordant for weight are the same

each trait simultaneously, not because one trait per se causes another Therefore, comparing identical twins who are discordant for a risk factor (e.g. food or drink preferences) provides more rigorous and informative insights on associations with health-related outcomes, than studies undertaken in unrelated individuals raised in different environments (Donovan & Susser 2011)

Greater liking of energy-dense foods or drinks is a hypothesised risk factor for the development of overweight; because preferences drive actual intake (Drewnowski et al. 1999; Biloukha & Utermohlen 2001; Glanz et al. 1998; Kourouniotis et al. 2016; Lennernäs et al. 1997). Results from **Study 2** demonstrated that these preferences are shaped by moderate genetic and non-shared environmental influences. Whilst discordance for BMI is rare in MZ twin pairs, it is this type of twin pairing which is particularly advantageous for identifying environmental influences that may be the cause or the consequence of overweight (Pietiläinen et al. 2004). Identifying environmental factors that increase the risk of overweight is particularly important as these are generally more easily modifiable than innate genetic factors.

Three previous studies have taken advantage of the genetically-sensitive twin design to examine the relationships between adult co-twins discordant for BMI and either food preferences (Rissanen et al. 2002), actual food intake (Doornweerd et al. 2016), or more recently, between discordance in body fat-distribution and dietary flavonoid intake (Jennings et al. 2017). Rissanen et al (2002) examined differences in food preferences between 23 pairs of adult MZ twins discordant for obesity (and differed in their BMI scores by >3 kg/m^2; age range: 35 – 60 years) using a semi-structured qualitative interview to assess liking for ‘fatty foods and ‘sweet foods’. The obese twins reported significantly higher preference for ‘fatty foods’. Another study explored differences in actual food intake between 16 pairs of adult MZs (mean age: 49.8 years) discordant for BMI (Doornweerd et al. 2016). The heaver twins had significantly higher intakes of monounsaturated fats, oils, and savoury sauces, measured using 24-h dietary recall. More recently, Jennings et al (2017) studied fat mass ratios (FMR, an indicator of adipose tissue distribution; calculated as trunk fat mass (kg)/limb fat mass (kg)) in MZ female twins discordant (>1SD) for flavonoid intake (phytonutrients predominantly found in fruit and vegetables), or the consumption of the main foods/drink sources that contribute to the intake of these micronutrients (Jennings et al. 2017). Dietary intake was measured using a validated 131-item FFQ. The co-twins (n=114-172) who reported higher intake of three flavonoid sub-classes (proanthocyanidins, flavan-3-ols, flavanols) were found to have significantly lower mean FMR values. In line with these results, the co-twins who differed significantly in their FMRs (0.03± 0.01) also differed in their habitual consumption of foods rich in flavonols (e.g. pears, tea, onions; p=0.01) or proanthocyanidins (e.g. apples and cacao; p=0.04).

Collectively, these studies suggest that higher liking of fatty foods is associated with higher BMI, and that higher intake of flavonoid-rich foods and drinks is associated with a favourable adipose-tissue distribution, independently of genetic- and shared environmental confounding. Previous studies of weight discordant twins have focused on investigating a very narrow spectrum of energy-dense foods, and neglected drink preferences, as potential risk factors for weight discordance. Because of this gap, it remains unclear to what extent preferences for a comprehensive range of food groups and drink types may be considered risk factors for weight gain when confounding for genetic and shared environmental factors is fully controlled for.

6.9 Study aims

The aim of this study was to compare food and drink preference between monozygotic twin pairs who are discordant for BMI to test the hypothesis that higher BMI scores are associated with higher preference for energy dense food and drinks, controlling for confounding by genetic factors as well as aspects of the environment shared by twin pairs (e.g. SES, ethnicity, age, sex). These factors can never be completely controlled for in studies of unrelated individuals. Additionally, associations between within-pair differences in food and drink preferences and within-pair differences in BMI will be explored across the whole sample of MZs (not just those with very discordant BMIs), to maximise power.

6.10 Methods

6.10.1 Sample

Participants for this study were 455 monozygotic twin pairs (910 individuals) from TEDS. Of these, 77 MZ twin pairs (154 individuals) discordant for BMI by ≥3 kg/m^2 were identified for discordant co-twin analyses.

6.10.2 Measures

6.10.2.1 Sociodemographic data

Age, sex and socio-economic status had been collected at baseline, with full details explained in **Chapter 3**. Zygosity status had been assigned using a 95% accurate questionnaire, and in cases of uncertain zygosity, DNA testing was used (Price et al. 2000) (again see chapter 3 for full details).

6.10.2.2 Food and drink preferences

The food and drink preference questionnaire described in **Study 1** was used to collect preferences for six food groups (vegetables, fruit, meat/fish, dairy, snacks, and starches) and seven common non-alcoholic beverage types (SSBs, Non-Nutrive Sweetened Beverages [NNSBs], fruit squash, orange juice, milk, tea, and coffee). Preference scores were collected on a 5-point Likert scale ('dislike a lot' to 'like a lot'), with higher scores indicative of higher liking.

6.10.2.3 Adiposity

Self-reported height and weight data were collected and BMI scores were calculated for each participant (kg/m^2). For this study, twin pairs were defined as BMI-discordant if BMI scores differed by at least three units (kg/m^2) between the twin and co-twin, in line with previous studies (Rissanen et al. 2002; Doornweerd et al. 2017; Pietiläinen et al. 2004).

6.10.3 Statistical analyses

Mean preference scores for each of the six food categories and seven drink types were calculated for the heavier and leaner co-twin of each BMI-discordant twin pair. Paired samples t-tests were used to identify significant differences in mean food and drink preference ratings, assuming equal variance across the co-twins (Altman 1999). Cohen's d was used to estimate the magnitude of the differences in preference scores across the heavier and leaner co-twin. Values of Cohen's d are interpreted as follows: 'small', 0.2 - 0.3; 'medium', ~0.5; 'large', >0.8 (Cohen 1988).

The 'paired-difference' method using all MZ pairs in the sample (n=455 pairs) was used to establish if larger within-pair differences in the liking of more energy-dense food and drinks were associated with greater BMI-discordance (Carlin et al. 2005). Difference scores for the food and drink preferences, and BMI indices, between twin pairs were calculated by subtracting scores for Twin 1 from Twin 2. The difference in BMI (the independent variable) was regressed against the within-pair difference in food and drink preference scores (the dependent variables) using a linear regression model. In recognition of the smaller sample size of the current sub-sample (nested within the larger TEDS sample used in study 3a), statistical significance was considered where p-values were reported as $p<0.05$. All analyses were conducted in SPSS Version 22.0 (SPSS Inc., Chicago, IL, USA).

6.11 Results

6.11.1 Summary statistics

Characteristics of the full MZ-only (n=455) sample and the BMI-discordant MZ twin pairs (n=77) are shown in **Table 6.6**. The mean age of the full MZ sample was 19.15 (SD=0.29), mean BMI was 22.29 (SD=4.36) and 61.5% of twin pairs were female. The BMI-discordant subsample had a mean age of 19.14 (SD=0.30), and was predominantly female (70.01%). Because of the pre-specified selection criteria, the leaner and heavier co-twins differed significantly for all anthropometric measures. BMI scores of the adiposity discordant twins differed on average by 5.56 kg/m^2 (SD=4.92). Mean BMI for the heavier twin (27.91 kg/m^2) was classified as 'overweight', while the mean value for the leaner co-twin (22.35 kg/m^2) was in the 'healthy' range (World Health Organization 2006). As expected, the weight of the heavier co-twins were also significantly higher than for their leaner co-twin (mean Δ: 12.97 (SD=12.18); t(76) = 2.47, p=0.008)). They also were significantly shorter (mean Δ: 3.31 (SD=10.05); t(76) = -2.889, p=0.005)). Effect sizes for these differences were medium to large.

Table 6.6 Demographic characteristics of the full MZ twin pair sample (n=455), and BMI discordant MZ twin pairs (n=77)

Characteristics[1]	Full sample	BMI-discordant MZ sample			
	All MZ pairs	**Heavier twin**	**Lighter twin**	**p-value[2]**	**Cohen's d[3]**
Age [mean (SD)]	19.15 (0.29)	19.14 (0.30)	19.14 (0.30)		
Sex [n (% M)]	175 (38.46)	23 (29.90)	23 (29.90)		
Weight [mean (SD)]	**64.35 (13.56)** range: 35.00 – 173.00	**78.94 (18.73)** range: 45.36 –173.00	**65.96 (12.89)** range: 35.83–102.06	**<.001**	**.81**
Height [mean (SD)]	**170.12 (10.35)** range: 101.60 - 208.28	**168.74 (12.00)** range: 101.60 - 201.00	**172.05 (11.20)** range: 152.40 - 198.12	**.005**	**.29**
BMI [mean (SD)]	**22.29 (4.36)** range: 14.12 – 59.74	**27.91 (6.95)** range: 18.89 – 59.74	**22.35 (4.27)** range: 14.12 – 35.19	**<.001**	**.96**

Significant findings are bolded.
[1] Weight reported in kilograms (kg), height reported in centimetres (cm), and BMI reported in kg/m^2
[2] Paired samples t-test for continuous variables
[3] Cohen's d is a measure effect size; Values for Cohen's d are interpreted as follows: 'small'=0.2 - 0.3; 'medium'~0.5; 'large' >0.8

6.11.2 Food and drink preferences in BMI-discordant MZ twins

In general, food and drink preferences were similar across BMI-discordant twin pairs (**Table 6.7**). Overall, the snack category was the most highly rated food group for all. Vegetables were the least liked food group in the heavier co-twins (mean: 3.61 (SD=0.73), whilst the dairy food group was the least liked group in the leaner individuals (mean: 3.62 (SD=0.81)), although only by a small margin. Tea was consistently the most preferred drink type and SSBs were the least liked for both the heavier and leaner co-twins. Food and drink preference scores between the BMI-discordant twins differed significantly for liking of fruit (t(74)= 0.135; p=0.036), such that the heavier twins (mean:4.26 (SD=0.74)) had a higher liking for fruit than their leaner co-twins (mean=4.05 (SD=0.96)). Paired samples t-tests for food and drink preference scores indicated that the heavier and leaner co-twins did not differ significantly for any other food or drink preferences

Table 6.7 Mean preference scores for food and drink categories among the heavier and lighter individuals of BMI-discordant identical twin pairs (n=77)

Food & drink category[1]	Mean score (SD) Heavier twin	Mean score (SD) Lighter twin	p-value[2]	Cohen's d[3]
Food				
Vegetables	3.61 (0.73)	3.64 (0.82)	.71	.04
Fruit	**4.26 (0.74)**	**4.05 (0.96)**	**.04**	**.28**
Meat/Fish	3.93 (0.70)	3.98 (0.59)	.58	.08
Dairy	3.64 (0.72)	3.62 (0.81)	.79	.03
Snacks	4.29 (0.73)	4.36 (0.66)	.37	.10
Starches	3.84 (0.70)	3.87 (0.71)	.77	.04
Drinks				
SSBs	3.53 (1.57)	3.49 (1.61)	.83	.03
NNSBs	3.78 (1.44)	3.71 (1.48)	.71	.05
Milk	4.15 (0.94)	4.31 (0.87)	.20	.17
Fruit squash	4.24 (1.04)	4.36 (0.97)	.38	.12
Orange Juice	4.27 (1.19)	4.39 (1.00)	.40	.11
Tea	4.43 (0.75)	4.62 (0.74)	.10	.25
Coffee	3.77 (1.45)	3.94 (1.31)	.55	.12

Significant findings are bolded.
[1] Comparison of group scores included observations from complete pairs only, with sample sizes as follows: Vegetables (n=75), Fruit (n=75), Meat/Fish (n=74), Dairy (n=75), Snacks (n=76), Starches (n=75), SSBs (n=74), NNSBs (n=73), Milk (n=67), Fruit squash (n=74), Orange Juice (n=74), Tea (n=53), and Coffee (n=31).
[2] Paired samples t-test were used to test for differences in continuous scores
[3] Cohen's d is a measure effect size; Values for Cohen's d are interpreted as follows: 'small'~0.2 - 0.3; 'medium'~0.5; 'large' >0.8
Abbreviations: SSBs=Sugar-sweetened beverages; NNSBs=Non-nutritive sweetened beverages

6.11.3 Paired differences analyses in all MZ twins

'Paired-difference' analyses were undertaken to identify if within-pair differences in food and drink preferences were associated with within-pair differences in BMI, in the full MZ sample to maximise power. Results are presented in **Table 6.8**.

Table 6.8 Linear regression coefficients for associations between within-pair differences in food and drink preferences and within-pair differences in BMI in all MZ twin pairs (n=455)

Δ Preference	Δ BMI				
	β	S.E	t-statistic	p-value	R^2
Food					
Vegetables	-0.200	±0.190	-1.053	.293	.002
Fruit	-0.033	±0.168	-0.194	.846	<.001
Meat/Fish	-0.217	±0.184	-1.179	.239	.003
Dairy	-0.074	±0.174	-0.423	.670	<.001
Snacks	-0.291	±0.233	-1.249	.212	.003
Starches	-0.143	±0.170	-0.840	.422	.002
Drinks	0.014	±0.087	0.162	.871	<.001
SSBs	-0.094	±0.090	-1.047	.296	.002
NNSBs	-0.229	±0.132	-1.730	.084	.007
Milk	-0.146	±0.118	-1.233	.218	.003
Fruit squash	-0.052	±0.111	-0.464	.643	<.001
Orange Juice	-0.036	±0.126	-0.288	.774	<.001
Tea	0.097	±0.119	0.816	.415	.003
Coffee	-0.200	±0.190	-1.053	.293	.002

[1]*Abbreviations: β coefficient = Unstandardized values of β; R^2=Coefficient of determination; S.E = Standard error, SSBs=Sugar-sweetened beverages; NNSBs=Non-nutritive sweetened*
[2] R^2 = Strength of the observed association; denotes the proportion of variation in the dependent variable that is explained by the independent variable.

Although mean liking for fruit had previously been shown to be significantly higher in the heavier co-twin compared to the leaner twin of a BMI-discordant pair, this difference wasn't significant when examining BMI differences across the whole sample. Within-pair differences for all six food categories and seven drink preference types were not significantly associated with within-pair differences in BMI in genetically-matched individuals.

6.12 Discussion

6.12.1 Summary of findings

The results from this study suggest that identical twins who are substantially discordant for BMI do not have markedly different food and drink preferences. The only significantly different preference was higher liking for fruit among heavier co-twins, but this within-pair difference was not associated with BMI-differences among the entire

MZ sample. After accounting for potential confounding by common genetic and shared environmental influences on both food and drink preferences and body weight, this study did not support the hypothesis that higher liking for energy-dense foods and drinks is associated with substantial adiposity differences in adolescent identical twins. In contrast to the findings in Study 3a, higher liking for dairy foods and NNSBs were not associated with higher BMI. It is possible that confounding by genetic or shared environmental influences may have contributed to the associations observed in the between-families analyses of unrelated individuals in Study 3. Despite this discrepancy, the findings from this study reiterate that relationships between food and drink preferences and adiposity are highly complex, and food and drink preferences may not be a key driver of body weight during emerging adulthood. The findings from this study provide new insights on the relationship between food and drink preferences and adiposity, unconfounded by genetic and shared environmental influences, and adds to a growing body of research attempting to identify environmentally-determined dietary preferences associated with adiposity gain.

6.12.2 Comparison of food and drink preferences between BMI-discordant twins

When the food and drink preferences of adiposity discordant identical twins were compared, only liking for fruit differed significantly. The liking for fruit was higher in the heavier twin, suggesting that greater liking for fruit is an environmentally-acquired food preference associated with adiposity. Previous literature assessing environmental lifestyle factors which differ between BMI discordant twin pairs has primarily focused on identifying differences in dietary *intake* (Doornweerd et al. 2016; Pietiläinen et al. 2010), limiting any direct comparisons. However, one longitudinal study of 152 MZ twin pairs investigating the association of dietary intake in childhood with weight gain trajectories into adolescence, found an opposing result with regards to the associations of fruit *intake* and BMI (Dubois et al. 2016). In this study, lower consumption of fruit at age 9 showed a continuing association with *higher* BMI during puberty. More importantly, comparison of dietary intake at age 9 between 48 MZ twin pairs that had been adiposity discordant ($\geq$2 kg/m^2) at least once during adolescence, found that dietary patterns of the heavier twins were characterized by significantly higher consumption of carbohydrates, fats, proteins, meat and meat alternatives, higher-fat meats *but less fruit* and fruit juice. This inverse association between higher consumption of fruit and adiposity is the opposite of the positive association between fruit liking and BMI in the current study. However, the mismatch of comparing intake and preference, as well as the differences in sample characteristics, is likely to account for some of the discrepancy between findings. Compared to dietary preferences, a wide range of psychological (e.g. lifestyle choices, personality), socio-cultural (e.g.

cultural norms) and situational factors (e.g. financial resources) mediate the influence of dietary preferences on dietary intake, and thus relationships between *intake* with BMI might provide different results (Köster 2009).

A further explanation for the observed inconsistency with regards to fruit and adiposity may be the cross-sectional nature of the data in the current study. While the co-twin control method provides a more in-depth understanding of the relationship of food and drink preferences with adiposity, independent of genetic predisposition, it cannot establish whether differences in food and drink preferences arose before or after the development of BMI-discordance. This question has been the topic of numerous previous studies which collectively suggest dietary preferences (especially for fatty or sweet-tasting foods) differ in obese and overweight individuals compared to normal weight controls (Reed et al. 1997). A recent review of the literature has summarized the evidence on the bidirectional relationship between food/taste preferences and weight status (Berthoud & Zheng 2012). The studies reviewed by Berthoud and Zheng (2012) suggest that there may be a gradual blunting of taste responsiveness in individuals with obesity, which may eventually encourage overconsumption of palatable foods – because they need to eat more in order to achieve the same taste satisfaction. On the other hand, in studies where individuals with obesity lose weight, this change in taste sensitivity was reversed. As of yet, the literature provides evidence in favour of a directional relationship, such that differences in taste sensitivity, palatability, and the reinforcing value of taste, can both be the cause and consequence of acquired obesity. Only one study of BMI-discordant twins has attempted to untangle the directionality of this relationship using a longitudinal study design. Rissanen et al found that recalled preference for fatty foods had arisen in early adulthood (at age 20-30) among 16 pairs of MZ twins, before the development of BMI discordance in later adulthood (Rissanen et al. 2002), implicating greater preference for high-fat foods as an environmentally-acquired driver of weight gain over time.

Taken as a whole, the finding that only one food preference type (fruit) was significantly different between the BMI-discordant twins challenges the initial hypothesis that liking for energy-dense food and drinks would be greater in the heavier co-twins. This raises the question of what factors do explain the observed BMI-discordance within the MZ twin pairs. Total energy intake is a crucial influence on adiposity but both energy intake and body weight are traits that are heritable and under the influence of social and cultural factors. For this reason, the BMI-discordant MZ twins design has been used to document the influence of other, perhaps more proximal, environmental lifestyle factors such as specific patterns of food intake on acquired obesity in six previous studies. Of these, four studies found that total dietary energy intake did not differ significantly within twin pairs (Dubois et al. 2016; Doornweerd et al. 2016; Naukkarinen et al. 2014; Pietiläinen et al. 2010). Of those that did not find a difference in energy intake between twins, two studies reported significant positive associations between higher intake of high-fat foods with BMI. In the study by Doorneweerd and colleagues (2016) total fat intake (% daily energy intake) recorded by 3-day dietary recall interviews was found to be higher in the heavier co-twin (n=16 MZ pairs). This finding is in agreement with a longitudinal study of 202 BMI-discordant twins which identified greater intakes of high-fat meat and milk in childhood, among the heavier co-twins, as dietary drivers of adiposity discordance in adulthood (Dubois et al. 2016). However, these results differ from a study of 14 MZ twin pairs discordant for obesity, which also found no difference in energy intake (recorded in 3-day diet diaries) between co-twins (Pietiläinen et al. 2010). Here, obese co-twins reported significantly lower consumption of mono- and poly-unsaturated fatty acids and lower intake of sweet and fatty 'delicacies' than their leaner co-twins. The fourth study reporting no difference in mean daily energy intake within twin pairs discordant for obesity was conducted using 3-day food diary in 16 adult MZ twin pairs, but failed to report more detailed information on differences in food intake patterns (Naukkarinen et al. 2014). A probable lack of power in this and the previous study may have limited the detection of small but important differences in dietary intake. Additionally it further underlines how unreliable dietary intake data are.

Both studies that reported significant differences in food intake between BMI-discordant twins relied on measures of 'food amount' rather than dietary energy intake (as used in the previously discussed studies). Food intake, as recorded by self-ratings of 'which twin eats the most?' most, was the greatest difference of all lifestyle factors in 174 MZ adult twin pairs discordant for BMI (van Dongen et al. 2015). Likewise, based on comparison of 713 MZ and 698 DZ twin pairs, the self-reported amount of food consumed by the heavier co-twin was substantially greater, suggesting that quantity of food consumed is an important environmentally-influenced contributor to obesity, independent of genetic predisposition (Bogl et al. 2009).

Overall, it appears that BMI differences between genetically identical subjects may be more strongly related to the amount of food consumed than to food and drink preferences, despite the substantial heterogeneity in study characteristics and findings. These discrepancies are likely the result of bias by the twins' perception of their weight difference, with the heavier twin systematically underreporting food intake (Pietiläinen et al. 2010). This specific underreporting in heavier twins relative to their leaner co-twin for dietary intake measures has been confirmed in a study where food records were validated with ‘gold standard’ doubly labelled water assessments (Pietiläinen et al. 2010). This may help explain why some twin studies fail to observe differences in energy and food intake between co-twins (Pietiläinen et al. 2010). On the other hand, it is also possible that underreporting of food intake is the result of conscious or unconscious under eating during a period of dietary assessment (Goris et al. 2000).

Still, studies of BMI discordant twins have failed to uncover consistent differences in dietary energy intakes between higher and lower adiposity co-twins, suggesting that other eating or physical activity behaviours may contribute to these differences. Irregular periods of increased energy consumption or dieting behaviours would not necessarily be captured by traditional measures of dietary intake. Limited evidence has been put forward which suggests heavier co-twins report more frequent dieting attempts, more restrictive eating behaviours, greater tendencies to overeat, higher feelings of hunger, greater cravings for sweet foods, and more body dissatisfaction than their leaner co-twin (Doornweerd et al. 2017; van Dongen et al. 2015; Keski-Rahkonen et al. 2004). Additional evidence comes from adiposity-discordant sibling studies. In a small study of 14 sibling pairs (aged 13-17 years) discordant for zBMI, within pair differences were found in delay discounting, with heavier siblings showing poorer gratification delay (Feda et al. 2015). Another recent study, investigated the relationship between aspects of an individual’s social network and variability in adiposity among sibling-pairs in the US (n=40 pairs; 13–17 years of age) (Salvy et al. 2016). Interestingly, friends’ zBMI score was the strongest predictor of participants’ zBMI, closely followed by SSB intake and time spent in sedentary behaviour. These findings support the idea that the unique social context is a salient factor in shaping BMI variability, and emphasizes that social factors potentially mediate the behavioural expression of food and drink preferences which contribute to discordance in adiposity of genetically similar subjects.

6.12.3 Findings on the relationship between paired differences in food and drink preferences and BMI-discordance

Comparing differences in food and drink preferences with paired differences in BMI across the whole sample of MZs revealed a seemingly contradictory relationship for liking of fruit. Greater liking for fruit was seen in the heavier twin, yet the magnitude of difference in the liking for fruit, did not contribute to the difference in BMI between twins when all MZs were considered. This could suggest that a greater liking for fruit may be a recently acquired consequence of higher BMI. Twin pairs, and especially identical twins are continuously subjected to comparison between each other throughout their life. It is possible that the heavier co-twin may be become increasingly conscious of this difference and may attempt to control their weight. Increasing fruit consumption, and thereby higher liking for fruit over time, may be the result of such a dieting attempt. This finding warrants a longitudinal follow-up study to ascertain if this difference in the liking for fruit between BMI-discordant twins predicts change in weight over time. Nevertheless, the actual difference in mean fruit preference scores between the heavier and leaner co-twin shows in absolute terms is minimal (heavier twins 4.26 (SD=0.74) vs. leaner twins 4.05 (SD=0.96)). Given the substantial size of the discordant MZ sample in this study (n=77), especially compared to the much lower sample sizes in previous studies of BMI-discordant twins (ranging from n=14 pairs in Pietiläinen et al. to the exceptionally large n=1311 pairs in Bogl et al (2009)), this statistically significant difference in means is likely the result of the high power in the present study design rather than an indication of a truly meaningful difference of phenotypic importance.

No differences in food and drink preferences were significantly associated with differences in BMI between co-twins, when the whole sample of MZs was analysed. However, this was expected given the small effect sizes seen in **Study 3a**. In addition, interpretation of these findings needs to consider that heritability of BMI is highest in young adults, with up to 90% of variability attributable to genetic factors (Dubois et al 2012). Previous research has also documented that BMI discordance in MZ twins is generally not a stable characteristic and that any large weight differences generally converge over time (Granér et al. 2012). Together these findings highlight the strength of genetic influence on weight maintenance (van Dongen et al. 2015). This could explain why food and drink preferences do not emerge as a key influence of BMI discordance in genetically-matched individuals.

6.12.4 Strengths and limitations

This study utilized the MZ twins from TEDS to examine associations between food and drink preferences with BMI, unconfounded by genetic and shared-environmental influences. By maximizing the use of genetically-sensitive data, it was possible to explore entirely environmentally-acquired food and drink preferences in one of the largest samples of young adult identical twins discordant for BMI.

However, a few limitations of the study must be acknowledged. Despite the unique study design, it was not possible to overcome the cross-sectional nature of the data. The food and drink preference questionnaire was completed at one time point and thus one cannot draw any conclusions on the temporal direction of food and drink preferences as causes or consequences of BMI-discordance. It will therefore be essential for future research to extend this discordant-twin design study to include longitudinal data. This approach has been successfully applied to follow growth trajectories of infants discordant for appetite, providing convincing evidence that differences in appetite are causal contributors to excessive early weight gain (van Jaarsveld et al. 2014). In addition, there could be other non-shared environmental confounding factors that lead to twins being discordant in both dietary preferences and BMI. For example, BMI discordance may be the result of unequal environmental exposure to food and drink occasions rather than a direct consequence of the obesogenic effect of certain food and drink preferences.

BMI was only measured at one time-point, so there was no indication of the stability of the discordance in BMI. Likewise, it was also not possible to consider whether food and drink preferences may have a gradual, cumulative effect on bodyweight over time, and thus only begin to express themselves phenotypically in later adulthood. This reiterates that future studies of a longitudinal design are needed to address the longer-term effects of food and drink preferences on adiposity.

The MZ co-twin control design does not eliminate the need to consider potential confounding of the observed findings. This is because MZ twins do not provide perfect matched controls as they cannot be matched for the unique wider environmental experiences that make them psychologically unique (McGue et al. 2010).

The wide range of food and drink preferences on the questionnaire enabled a comprehensive range of preferences to be considered, not only for ‘high risk’ foods (usually energy-dense groupings of ‘fatty’ and high sugar items), but also for potentially protective preferences in relation to adiposity discordance. Also, and perhaps most importantly, this was the first study to separately consider drink preferences in BMI-discordance MZ twins, an area of dietary preferences which has never been studied before.

It is also important to bear in mind that no data on other important lifestyle or eating behaviours were considered which may have contributed to the observed discordance in BMI (e.g. physical activity, other eating behaviours, eating disorders). This is also true for other non-behavioural dimensions such as desire for a specific body type, which may be crucial in influencing the relationship between food and drink preferences and outcomes such as body weight. More generally, the discordant twin design can only be used to study non-shared environmental risk factors. The heritability of BMI is high, with up to 50-90% of variation being explained by genetic influences (Dubois et al. 2012). For this reason, BMI discordance in MZ twins is particularly rare and final analyses were conducted in a relatively small sample of 77 MZ twin pairs. However, sample sizes of <20 pairs are typical for these types of studies (Doornweerd et al. 2017; Rissanen et al. 2002).

The findings also need to be interpreted keeping the usual limitations of self-reported measures in mind. There may have been under estimation of weight at the higher end of BMI, potentially biasing validity of the BMI measure. In addition, social desirability may have also biased dietary preference ratings and weight data, such that participants (especially the heavier twin of a pair) may have under-reported their weight and over-reported the liking of foods and drinks generally considered healthier. In relation to this, there are some further disadvantages of the discordant co-twin study design which need to be considered in the interpretation of the results. The comparison of food preferences among co-twins might be biased by the twin's perception of their weight difference, with the heavier twin possibly under-reporting their true preferences (van Dongen et al. 2015). However, preferences were measured via an anonymous online questionnaire when the twins were not necessarily in each other's presence, so the risk of this bias was minimized. Another limitation is whether MZ twin pairs are generalizable to the wider population, however a comprehensive pooled analysis of 11 twin studies found no concern relating to this (Bouchard Jr. 1997). Nevertheless, the sample consisted of predominantly healthy, white, lean, and young a participant which means the findings cannot necessarily be extrapolated to the general population.

This study was also not able to consider differences in prenatal in-utero experiences or epigenetic factors which may have contributed to slight differences in the metabolic health and obesity risk of one twin over the other (Czyz et al. 2012; Fraga et al. 2005). Irrespective of these limitations, the discordant MZ-twin method offers a unique tool to establish whether a probable causal pathway between food and drink preferences and BMI exists, grounded on the assumption that the influence of genes and shared environmental experiences are controlled for in the discordant MZ-twin method. The results provide useful insights to inform the development of effective obesity prevention programs.

6.13 Conclusions and implications

In spite of some of the limitations, this study used a co-twin control design to establish that no foods or drink preferences are associated with greater risk for adiposity, after controlling for genetic and shared-environmental confounding. Overall, food and drink preferences are similar for BMI-discordant identical twins, supporting the conclusion that food and drink preferences are not environmental risk factors associated with greater adiposity during emerging adulthood. This suggests that other environmental factors in a young adult's own environment affect the relationship between food and drink preferences and actual food intake: other environmentally influenced behavioural patterns, such as eating styles and portion sizes merit further study. While the underlying contribution of food and drink preferences to adiposity appears minimal, future research needs to address the inadequacies in the measurement of both food and drink preferences and adiposity in population-based samples to replicate the findings seen in this study.

This conclusion agrees with the findings that food and drink preferences only explain a small amount of variance of BMI in the full TEDS sample, as observed in Study 3a. Adoption of healthy lifestyle behaviours (e.g. physical activity) or exposure to other unique environmental influences (e.g. unshared friends), may be responsible for BMI discordance in identical twins. Development of new approaches to prevent excess weight gain should aim to identify other environmental factors responsible for BMI discordance in genetically-identical individuals. In conclusion, body adiposity discordance in genetically identical individuals may be shaped more by the '*how much'* or *'why'* rather than the '*what*' of young adult's food and drink choices.

Chapter 7.

Study 4: Sweetness preference modification intervention in relation to hot beverages in young adults – The REduction of Sugar In tea STudy (RESIST)[I]

7.1 Introduction

In **Studies 1, 2** and **3**, observational methods were used to establish the aetiology of a wide range of food and drink preferences and cross-sectional relationships with adiposity. Both food and non-alcoholic drink preferences were found to be under considerable genetic influence with the remaining variation in these preferences shaped by aspects of the environment that are not shared by twin pairs (e.g. influences outside the family and home). Non-shared environmental factors appeared to be of greater importance for drink preferences than food preferences, indicating these preferences may be more amenable to environmental modification. **Studies 3a** and **3b** found food and drink preferences are not cross-sectionally associated with adiposity in late adolescence. However, this was a lean sample and greater liking for energy dense and 'unhealthy' food and drink may not yet have had sufficient time to influence adiposity by this age, whereas other health behaviours may be more proximal factors influencing weight management.

This chapter will briefly review the current literature on food and drink preference modification. Based on the identified gaps in the literature, **Study 4** will present the rationale, design and results of a preference modification intervention to reduce liking for sweetness in hot tea.

[I] Data from this chapter were presented on the 9th of November at the Sugar Reduction Summit 2017 in London, UK. The presented findings received the highly commended award for 'Best Research in Sugar Reduction or Sugar Alternatives' at the 2017 Sugar Reduction Awards.
Smith, A.D., Fildes, A. Cooke, L. and Llewellyn C. - The REduction of Sugar In tea STudy (RESIST): a pilot randomised trail comparing the effectiveness and feasibility of gradual versus immediate cessation to reduce sugar intake in tea
A copy of this poster is provided in **Appendix F15.**

7.2 Background: Modification of food and drink preferences

7.2.1 Dietary preference modification

Taste preferences acquired early in development have important implications for nutrition-related chronic disease risk later in adulthood (De Cosmi et al. 2017). Large public health gains could be achieved if preferences are successfully shifted towards more healthful choices (Nicklaus et al. 2005).

A large body of published evidence from the consumer acceptance literature has assessed the impact of changes in sensory properties on perceived hedonic acceptance and palatability of food or drink (Mccrickerd & Forde 2016). When tastant intensity of a food is noticeably altered to a less preferred level this can affect subjective appetite and overall energy intake (Yeomans 1996). For example, in an experiment of 54 volunteers, palatability of a pasta dish was manipulated by altering the concentration of oregano flavouring (0.0%, 0.27% and 0.54%) in the pasta sauce. Three different intensity levels were tested within the context of a single eating occasion, with the meal being divided into three separate eating episodes. During each eating period more oregano was gradually added to the pasta sauce. The addition of oregano to a palatable level (0.27%) resulted in increased pasta consumption. However, food intake decreased significantly once the palatability level was exceeded, emphasizing the importance of taste properties on the control of energy intake.

In a comprehensive review on the influence of palatability on appetite and energy intake, Sørensen et al concluded that published evidence on the whole supports that intake of a previously bland food will increase if it is made to taste more palatable by adding sweetness, saltiness or various spices (Sørensen et al. 2003). Importantly, individual taste preferences have repeatedly been identified as important factors in explaining this effect. Healthy young adult volunteers (n=64; 18-35 years) who reported a greater liking for sweet foods compared to savoury foods ate more of sweet rice dish compared with a savoury risotto alternative. The same was true for participants that reported greater liking of savoury foods; intake of the savoury dish exceeded that of the sweet version (Griffioen-Roose et al. 2009). Moreover, volunteers who did not differ significantly in their pleasantness ratings between the sweet or savoury dish were shown to consume a similar amount of both versions. The insights from this study suggest that taste does not influence satiation directly, independent of overall palatability, texture and energy intake.

However, there is further evidence which shows that individuals can also gradually accept alterations in pleasantness of foods without affecting total food intake in the medium term. In a small five day cross-over study of healthy adults (n=35), daily repeated exposure to a less preferred food (i.e. low salt bread), resulted in no change of self-rated pleasantness or saltiness of that food, yet the desire-to-eat and intake increased slightly (Zandstra et al. 2000).

Further research also emphasises energy density as an important quality of food which influences dietary preferences (Wardle et al. 2003). In a study of 4-5-year-old UK-born twins (n=416), mean liking scores for 21 fruit and vegetables significantly increased with energy density.

Foods of higher energy-density likely have a higher fat content, which affects hedonic and sensory properties of food, and signals dietary energy to the consumer. One 12-week trial attempted to find out if adoption of a fat-restricted diet diminishes the hedonic ratings for high fat foods. In this three-armed trial, a group of 27 volunteers were randomised to either a restrictive very low fat diet (<20% of total energy intake), a low fat modified discretionary fat diet, or a control group (Mattes 1993). There was a substantial decrease in the mean hedonic ratings of both regular and the reduced-fat test foods (rated on a 9-point hedonic Likert scale) for participants in the low-fat diet condition, after completion of the 12 week active diet period. The authors concluded that repeated exposure to altered levels of dietary fat reduced the preference for fat in the short term. However, during three month follow-up, it was shown that this hedonic shift did not affect long term fat consumption as all three groups had returned to baseline habitual fat intake (assessed by 7-day diet diaries at baseline and throughout the study) (Rolls 1994). Moreover, studies on consumer preferences have also shown that repeated daily exposure (over 12 weeks) to reduced fat versions of potato crisps or cheese does not affect sensory or hedonic responses to these products (Mela et al. 1993). At present, evidence on successful shifting of fat taste preference as a direct consequence of dietary manipulation still remains inconclusive (Tuorila 2000).

Overall, the evidence above highlights the wide range of study methods used to assess the modifiability of dietary taste preferences and the subsequent impact on food liking or food intake. Larger longitudinal studies are necessary to capture and comprehend the dynamic nature of hedonic responses to sensory property modification these insights will help to gauge the possible effectiveness of taste preferences modification as a viable intervention targets to improve dietary choices and weight management.

7.2.2 Food and drink preference modification in children

Interventions aimed at changing preferences for foods and drinks, rather than intake, have almost exclusively been undertaken in children, perhaps because their behaviours are considered more malleable than adults. Beyond the innate affective response towards basic tastes such as sweetness and bitterness, likes and dislikes for foods and drinks are also learned via a variety of different mechanisms (Rozin & Vollmecke 1986). Generally, repeated taste exposure is recognised as one of the most important learning models for the acquisition of food and drink preferences. Exposure to a taste increases familiarity, which in turn increases food acceptance, which eventually translates to changes in ingestive behaviours. Findings from a number of previous food preference modification interventions targeted at children demonstrate that repeated exposure to a taste can result in increased liking and intake of a previously unknown or disliked fruit or vegetable (Caton et al. 2013; Anzman-Frasca et al. 2012; Remy et al. 2013; de Wild et al. 2013; Fildes, van Jaarsveld, Wardle, et al. 2014; Maier et al. 2007; Wardle et al. 2003). The addition of a conditional reward component (e.g. a sticker) to the exposure protocol was found to further increase effectiveness and maintenance in three similar food modification intervention studies in young children (Cooke et al. 2011; Remington et al. 2012; Corsini et al. 2013).

7.2.3 Food and drink preference modification in adults

At present, no equivalent behavioural intervention to increase the liking of food or drinks has been undertaken in adolescents or adults. However, indirect evidence for the plasticity of adult taste preferences can be inferred from nation-wide public health initiatives such as the UK Food Standards Agency's salt reduction programme (Wyness et al. 2012). From 2003 to 2010, the UK population's salt intake was reduced as a result of a progressive reformulation strategy, implemented by food manufacturers on a voluntary basis. This so called 'stealth' approach effectively re-sensitized palates to lower salt levels, suggesting that the UK adult population has adjusted to lower salt concentrations (He et al. 2014).

While no explicit interventions have studied the effectiveness of a targeted food or drink preference modification program in adult subjects, a body of literature has examined the modification of preferred *taste* intensity (e.g. salt or sweetness intensity). Current understanding of shifting *taste* preferences (not *food* preferences) is informed by studies that have demonstrated a strong effect of dietary experiences in establishing preferred levels of saltiness in food and drink (Bobowski 2015). For instance, there have been five studies that have demonstrated that preferred salt levels in food and drink can be modified through exposure to diets high or low in sodium. Three of these studies instructed participants to maintain reduced sodium diets (for 5-12 months) and assessed saltiness intensity ratings and pleasantness ratings of saline samples pre- and post the intervention period (Bertino et al. 1982; Blais et al. 1986; Elmer 1989). Participants in all three trials experienced significant decreases in preferred saltiness intensity and preference ratings in saline samples at the end of the study periods. Further support for the association between alterations in dietary salt intake and salt preference intensity were seen in the two other trials which demonstrated a significant increase in salt preference levels after dietary sodium consumption was increased unbeknown to the study participants (Bertino et al. 1986; Huggins et al. 1992). To identify strategies to improve population-wide diet quality, establishing the malleability of preferred levels of tastants (e.g. salt or sugar) within familiar food or drink items is becoming increasingly important (Stamler 1997). This is because consumption of salt and added sugars vastly exceeds the recommended levels in the UK, and the resulting impact on healthcare costs are large and steadily growing (Department of Health 2011). Establishing that modification in tastant concentration can affect changes in dietary preferences provides relevant evidence to push for policy change and industry reformulation efforts to lower salt and sugar in our food systems to levels consistent with optimal health.

In two trials, it has been experimentally shown that liking for a reduced-sodium version of a single food (i.e. soup) or beverage (i.e. tomato juice) can be altered without manipulation of sodium-levels of the entire diet (Bobowski et al. 2015b; Methven et al. 2012). In the trial investigating modification of sodium preference levels in soup (n=37; mean age: 32.8 years), an increase in liking for a lower sodium soup recipe was seen surprisingly early in the exposure period of eight successive working days. Encouragingly, the increase in preference levels peaked at day 3-5 in participants allocated to the low-sodium soup condition, and lasted for the duration of the trial. The observed change suggested that acceptance of a reduced-sodium version of a food can be achieved by means of repeated incidental exposure even in the context of an otherwise unchanged diet (Methven et al. 2012). The efficacy of two salt reduction strategies to increase liking of a lower sodium tomato juice was compared in the second trial. In this repeated exposure trial, adult volunteers (n=83) were allocated to either an abrupt or a gradual reduction condition in which they were required to consume a low or reduced sodium version of the same tomato juice three times a week for a 16-week time period (Bobowski et al. 2015b). Liking for the reduced sodium samples of the tomato juice increased significantly for both conditions equally post-intervention yet this was not accompanied by an increase in perceived saltiness intensity in either condition. Overall, these findings complemented the results from the Methven et al (2012) trial by providing further evidence that experimental manipulation of salt concentrations in a single familiar dietary item, independent of any other dietary changes, successfully modified preference. Importantly, in the context of sodium in tomato juice, there was no significant difference in intervention success between the different approaches to sodium reduction. Overall, replication studies will be necessary to confirm to what extent these findings apply for other tastants besides salt, and for other food and drink items.

However, research into the modification of other taste preferences beyond saltiness is sparse. One experimental study investigated the effect of repeated exposure to sweet or sour orangeade on taste preferences in a sample of adults and children (Liem & de Graaf 2004). Preference for the sweet orangeade increased significantly in the children (n=59; 6-11 years old) over the course of the 8-day repeat exposure intervention, but no changes in sweet or sour preferences were seen in the adults (n=46). This suggests that exposure-based taste preference modification in adults may require a higher number of, or more frequent, exposures compared to children (Johnston & Foreyt 2014). However, this hypothesis needs to be tested in a robust randomised controlled trial.

Only two randomized controlled trials (RCT) in adults have attempted to establish the effectiveness of sweetness preference modification. In the first trial, adults were recruited and randomized to an intervention or control group for a four month long experimental procedure (Wise et al. 2016). The intervention group (n=16) was assigned to a low sugar diet (replacing 40% of calories from sugars with fats, protein and complex carbohydrates), while the control group (n=17) maintained their habitual diet for three months. In the final month, both groups were instructed to eat as they wished. Once a month, both groups were asked to rate perceived sweetness intensity and palatability of a sweetened vanilla pudding and raspberry flavoured lemonade. Sweetness intensity was measured using a labelled magnitude scale (gLMS), anchored by descriptors ranging from 'barely detectable' to 'strongest imaginable sensation of any kind'. Sweetness pleasantness was rated on a 23-point scale ranging from 'very unpleasant' to 'very pleasant'. After the second month on the low sugar diet, participants in the low sugar group rated the sweetness intensity significantly higher than the control group. After the third month, sweetness intensity was perceived as comparatively higher for both the pudding and the raspberry drink in the intervention group. However, there was no effect on perceived *preference* for either the pudding or the raspberry drink throughout the three months of the intervention. While this suggests sweetness sensitivity changes as a result of diminished dietary exposure to sugar, it also proposes that the mechanisms regulating sweetness preferences and sweetness sensitivity may operate via different bio-behavioural pathways.

The second RCT investigating sweetness preference modification compared two theory-based sugar reduction techniques to support coffee drinkers in reducing the amount of added sugar without decreasing the enjoyment of coffee (Lenne & Mann 2017). Participants were randomized to one of three conditions, including two intervention conditions: one a gradual reduction of sugar in coffee group (n=46), and the second condition a mindfulness-based condition in which participants (n=43) received professional training at baseline in behavioural techniques to acquire an appreciation for unsweetened coffee over the intervention period. These conditions were compared to a 'repeated exposure' control group (n=40), instructed to cut out sugar in coffee without the provision of further guidance on how to implement this behaviour. On each day of the 14-day intervention, adult participants were asked to keep a record on their mobile phone of the number of cups of coffee consumed, the percent of coffees consumed that were sugar free, and daily enjoyment of the first sip of coffee. Daily enjoyment of coffee was measured using two scales, averaged into an overall rating. On a 7-point Likert scale ranging from 'Not at all' to "Very Much", participants were asked to indicate how much they agreed with: 'How much do you like your coffee?" and "How unpleasant does your coffee taste?". All three conditions were effective in cutting down the amount of sugar in coffee, and in maintaining this change up to 6-months post intervention completion. After completion of the intervention period, the mindfulness condition was most effective; participants consumed an average of 53 fewer sugar kcal/day, closely followed by the control group with 36 fewer sugar kcal/day, and participants in the gradual reduction group saved on average 21 sugar kcal/day. During the intervention, participants in the gradual reduction experienced a steady decline in perceived enjoyment of coffee, while participants in the mindfulness and control group showed a substantial increase in enjoyment of their unsweetened coffee. The increase in acceptance of the unsweetened coffee in the mindfulness intervention group however did not exceed the effect seen in the control group. Overall, the results from this trial indicate that gradual reduction of sugar in coffee is not effective in modifying liking for unsweetened coffee over a 2-week period, and that giving up sugar in one go (accompanied with or without a mindfulness program) may be more effective in achieving lasting elimination of sugar from a hot beverage.

A further attempt to assess the feasibility of adult palate re-education was investigated in a 'Two-week sugar challenge'. In this study, an opportunity sample of 20 adults was instructed to cut out all added sugars and artificial sweeteners in their habitual diet (Bartolotto 2015). After the 14 days of the challenge, 95% of the study participants reported that sweet foods and drinks tasted 'sweet or too sweet'. Interestingly this resensitization of sweetness preference levels appeared to extend to other types of food too, with 75% of participants reporting that perceived sweetness of foods such as carrots, apples and crackers tasted sweeter after completing the challenge. These results suggest that a self-imposed reduction of sugar and sweetener consumption has the potential to reduce preferred natural sweetness levels in food and drinks.

7.2.4 The Incidental Taste Memory Model – A conceptual framework underlying the learning and modification of food and drink preferences

The Incidental Taste Memory paradigm provides an extension to the explicit ‘mere exposure’ theory which has been the focus of most pervious taste modification studies, primarily undertaken in samples of young children. ‘Mere exposure’ trials mainly focus on the conscious modification of taste preferences by gradually increasing familiarity to a food item, which over time develops into greater preference (Zajonc 1968). In contrast, central to the Incidental Taste Memory model lies the hypothesis that preference for a food or taste are incidentally learned and dependent on the context within which they are learned. In other words, taste preferences are an expression of a learned memory for a previously experienced taste, and alteration of taste memory can alter taste preference (Köster 2009). For example, in the context of a diet which includes multiple cups of sugar-sweetened tea daily, a slight reduction in habitual sweetness in tea will register as less enjoyable whereas in the context of a diet which usually comprises unsweetened tea, an increase in sweetness is perceived as less pleasant. In that sense, the ‘incidental taste memory model’ builds on the mere exposure paradigm by explicitly considering the role of incidentally-learned taste norms as well as considering the context of active taste preference manipulation. It further suggests that any unnoticed changes to the taste of a food can progressively re-set expectations (or ‘memory’) of taste norms and food preference over time.

In a series of ten seminal studies, researchers studied various characteristics (age, gender) and sensory aspects of food or drink (flavour intensity, taste, texture, aroma and novelty) to explore which factors influence (incidental) taste memory in a normal eating situation (Laureati et al. 2008; Møller & Hausner 2006; Møller et al. 2007; Sulmont-Rossé et al. 2008; Morin-Audebrand et al. 2009; Morin-Audebrand et al. 2012; Mojet & Köster 2002; Laureati & Pagliarini 2013; Köster et al. 2004). Usually under false pretence, study participants are invited to have a meal in a research department, and then given a distraction task and not explicitly instructed to remember anything about the food they are presented. These studies found no effect of age on taste memory; the strength of the incidental taste memory performance did not degrade with age (Laureati et al. 2008; Møller & Hausner 2006; Møller et al. 2007; Sulmont-Rossé et al. 2008; Morin-Audebrand et al. 2009). Effects for gender were mixed, with two studies finding females' taste memory performance to be superior to males (Møller & Hausner 2006; Laureati et al. 2008). However, upon merging and re-analysis of six taste memory studies, the pooled effect of gender was no longer significant (Morin-Audebrand et al. 2012). These studies suggested that characteristics such as flavour intensity (Møller et al. 2007), basic tastes (Köster et al. 2004; Møller & Hausner 2006; Laureati & Pagliarini 2013) and texture (Mojet & Köster 2002; Laureati et al. 2008) can be unconsciously retained. Notably, memory for sensory aspects of food was not a universal phenomenon, and acuity in memory varied depending on the sensory aspect and the product, e.g. sweetness intensity of a cherry custard is better remembered than texture or aroma (Morin-Audebrand et al. 2009). Even within the same sensory parameter (e.g. basic taste) precision of food memory varied; deviation from remembered sourness (in orange juice and yoghurt) and bitter tastes were more precisely remembered compared to changes in sweetness (in orange juice and yoghurt) but this was once again product-dependent (Köster et al. 2004). Similarly, upon comparison of incidentally learned food memory across solid (biscuit), semi-solid (fruit purée), and liquid (fruit juice) target stimuli, strength of taste memory for sweetness intensity was strongest for the semi-solid fruit puree. These results further implied that acuity of taste memory is not only product-dependent but also related to the food structure (or 'food matrix') (Laureati et al. 2011).

However, the most consistent effect was seen for novelty and change detection in any aspect of food or drink. Taste memory appears most sensitive at detecting change from a previously experienced taste, suggesting that memory is strongly modulated by novelty (Morin-Audebrand et al. 2012). The extent to which some sensory characteristic deviates from what is anticipated for a food or drink (e.g. sweetness in a dessert, a smooth texture of yoghurt), appears to be the most significant determining factor influencing the strength of change in incidental food memory. Thus, the same sensory food aspect is remembered to the degree that it would be expected in the food-context in which it is encountered (Laureati et al. 2011).

Collectively, these studies provide evidence of the substantial unconscious aspects of food exposure and dietary experiences (texture, novelty detection, taste intensity, aroma) that shape an individual's food and drink preferences. Because these preferences are not consciously learned, they may be particularly difficult to modify by cognitive messaging or health promotion campaigns. Even though people are usually aware of their unhealthy food habits (e.g. snacking in front of the TV or adding sugar to tea), the habitual nature of these behaviours makes them challenging and unpleasant to change. Quitting unhealthy behaviours is suggested as an effective strategy to re-condition behaviours and to avoid daily temptation to fall back on old behaviours. Yet such a drastic approach requires a great deal of cognitive effort and may result in other negative compensatory behaviours (e.g. quitting snacking in front of the TV but having a slice of cake for dessert) (Kwasnicka et al. 2016). However, the incidental food and taste memory model proposes that it may also be possible to reverse unhealthy preferences using step-wise sensory “re-learning” programs (Köster 2009). A gradual approach may be a more pleasant and practical way to entice some people to modify their dietary preferences. Effectiveness and acceptability of such an approach however needs to be tested experimentally.

1.1.1 The need for food and drink preference modification trials in adolescents and young adults

There is a distinct lack of behavioural food and drink taste preference modification interventions targeting adolescents or young adult populations. This is problematic given that young adults are an important target population for health promotion and chronic disease prevention campaigns (Nelson et al. 2008). Late adolescence often involves a move away from the family home for the first time which can result in the adoption of unhealthy lifestyles or poor food habits (Arnett 2000). At the same time, the food and drink industry reinforces this tendency by strongly targeting young adults with sophisticated marketing strategies to promote the consumption of palatable but unhealthy dietary choices. Accordingly, unhealthy lifestyles at this age are associated with disease risk in the long term (Spring et al. 2014). Additionally, because dietary behaviour change is notoriously challenging, it has been suggested that modification of dietary preferences should be best undertaken at key re-orientation periods in life (e.g. retirement, pregnancy or emerging adulthood). These phases are considered developmentally sensitive periods and provide the opportunity for lasting preference change (Köster & Mojet 2007).

Young adulthood provides a unique window of opportunity for intervention. This period of development is marked by the re-organisation of self-identity and the development of self-efficacy – both important attributes that support the formation of long-lasting health behaviours. The stronger an individual considers a healthy lifestyle to be part of their identity, the more likely it is for such health-promoting behaviours to persist (Miller et al. 2002). Furthermore, interpersonal influences and support systems evolve, meaning social and other external networks surpass the home environment as the most important influences on behavioural patterns of young adults. Taken together, the unique characteristics of young adulthood make it an ideal time for establishing lifelong adiposity- and health protective behaviour change, but it is frequently overlooked in intervention research (Nelson et al. 2008).

7.2.5 Rationale for targeting the reduction of liking for sweetness in hot beverages (tea) in young adults

Free sugar ingested from any drinks stand out as especially harmful to cardiometabolic health and energy balance, as the energy ingested from beverages is not compensated for by an equivalent reduction in food intake (DiMeglio & Mattes 2000). Longitudinal evidence from a study of youths at high risk of obesity (n=564; aged 8-10 years) linked consumption of added sugars from liquids but not from solids with impaired glucose homeostasis and greater risk of insulin resistance over a 2-year period (Wang et al. 2014). Recently, higher sugar intake measured objectively was significantly associated with higher BMI and a greater odds of obesity in the UK (Campbell et al. 2017). In response to the burgeoning evidence that free sugar intake is a leading contributor to over-nutrition, dental caries and type 2 diabetes, the WHO guidelines were updated to recommend limiting sugar intake to 5% of daily energy (as opposed to the 10% previously recommended) (World Health Organization 2015).

Drink consumption patterns have changed considerably over the last few decades with a decrease in milk consumption and a marked increase in SSB intake. Drinks account for 14% of daily energy intake for 4–18 year olds and 18% of daily energy intake for adults (Ng et al. 2012). In the US, beverages account for 20% of all energy intake, with 35% of these calories attributable to intake of soft drinks. More specifically 47% of all added sugar in the average US diet (population >2 y) comes from beverages, and added sugar in tea and coffee account for 7% (US Department of Health and Human Services 2015). The widespread and frequent consumption of hot beverages such as sweetened teas and flavoured coffees are a neglected and easily underestimated source of free sugar intake.

Population level data from the US found that drinking daily coffee (n=13185) or tea (n=6215) with caloric add-ins (e.g. sugar or milk), was associated with greater daily energy intake of 69 kcal/day (42 kcal/day from added sugar) from coffee and 42 kcal/day (37 kcal/day from added sugar), from tea (An & Shi 2017). These figures however are likely conservative as these prospective data were collected using 1-day dietary recalls which are liable to social desirability bias, as study participants systematically underreport sweetened beverage intake. In addition, these findings were based on secondary data (the National Health and Nutrition Examination Survey [NHANES]) and it was not possible for the authors to consider caloric additives already contained in pre-blended sweetened coffees and teas. In a recent report by Action on Sugar, 55% of the 131 hot drinks surveyed from coffee shops across the UK contained the equivalent of, or more than, the total daily recommended adult sugar intake (Action on Sugar 2016). Coffee shop visits are a daily habit for a large proportion of the population, with about 20% of adults and adolescents reporting a daily purchase in one of the approximate 18,000 outlets across the UK (The Financial Times 2015). Additionally, around 70 million cups of coffee and 165 million cups of tea are consumed every day in the UK (Mintel 2008; UK Tea and Infusions Association 2016). While the sugar added to hot beverages is usually less than would be found in lemonades or other soft drinks, the fact that many people drink numerous cups of tea and coffee throughout the day, means that these small amounts of sugar rapidly accumulate.

Reducing sugar intake is a necessary population health aim, but foods and drinks high in sugar are highly palatable and people find it challenging to decrease their consumption of these. Sweetened drinks may also habituate individuals to sweet tastes, gradually increasing sweetness preference thresholds (Bartolotto 2015). While sugar plays an important role in the taste, texture and shelf-life of foods, the influence on texture and mouth-feel for drinks is less noticeable. This means it is technically easier for drink manufacturers to reduce or eliminate sugar from drinks without affecting mouth-feel (in contrast to fat in food items, for example). Evidence from multiple intervention studies in children suggests that preferences for foods and drinks can be changed, but this idea remains largely unstudied in young adults or adulthood. At the same time, large proportions of young adults are consumers of hot drinks (such as tea), and these are often sweetened with sugar (Ng et al. 2012). Altering incidentally learned taste expectations of sweetness within the context of a single drink may be a viable and effective approach to reduce intake of and preference for sugar in tea. Targeting the reduction of added sugar in a habitually consumed beverage may aid young adults in reducing their intake of free sugars overall, thereby identifying a feasible lifestyle change to reduce the risk for the health issues associated with high level of sugar consumption. Developing an intervention to support young adults in decreasing their preference for added sugar in their tea may play a useful role as part of a multi-component public health strategy to lower sugar consumption in line with recommended levels (World Health Organization 2015).

7.3 Study aims

The key objective of the '**RE**duction of **S**ugar **I**n tea **St**udy' (RESIST) is to pilot a new sweetness preference modification program aimed at reducing preferred sweetness levels for hot tea in young adults. This intervention is based on the incidental taste preference learning mechanisms identified as crucial to taste modification (Mojet & Köster 2002; Mojet et al. 2005; Morin-Audebrand et al. 2009). (Mojet & Köster 2002; Mojet et al. 2005; Morin-Audebrand et al. 2009). As indicated in the literature review, gradual modification of dietary intake has the potential to alter food preferences, as well as intake. Similarly, evidence from the salt reduction literature indicated that step-wise reduction of salt-levels in the food supply was highly effective in achieving sustained reductions in preferred salt levels at the population-level and in highly motivated individuals. However, an immediate cessation approach is overall more effective in achieving quit success for a habit such as smoking, as individuals are not continuously exposed to the active stimulus-reward association, resulting in maintained reliance on the stimulus (sugar) and cravings (for hot sweetened tea) (Shariff et al. 2016; Lindson-Hawley et al. 2016). Therefore, the RESIST trial was designed to test the hypothesis that sugar reduction via progressive, step-wise reduction to a level of no-added sugar in tea would prove more effective for achieving and maintaining this lower level of sweetness level in the context of a single beverage, compared to a sugar reduction protocol guiding the individual to make this transition in one step. Moreover, the trial will set-out to test the feasibility, preliminary effectiveness and acceptability of decreasing sweetness preference in tea by instructing participants to either gradually reduce or completely cease current intake of sugar in tea to reduce incidentally learned 'norms' for preferred sweetness levels in tea.

Research aims for **Study 4** were centred on evidence-based recommendations for good practice in relation to the design of pilot and feasibility studies (Lancaster et al. 2004). In line with these recommendations, the specific objectives of the RESIST trial were:

<u>Development of intervention</u>

1) To develop a low-cost and simple intervention package for the RESIST protocol

<u>Feasibility</u>

2) To estimate general interest for the intervention, rates of recruitment and rates of consent.
3) To test functionality of the study components and materials.
4) To test the integrity and smoothness of the study protocol for a future trial

<u>Effectiveness</u>

5) To test and compare preliminary effectiveness of a gradual reduction versus immediate cessation of sugar in tea, in achieving total elimination, or reducing, sugar in tea and altering preferences for sweetness intensity.

6) To inform selection of the most suitable primary outcome measure(s).

7) To gain initial estimates of the standard deviation of outcome measures to estimate sample size for a fully-powered trial of the RESIST protocol

Acceptability

8) To assess acceptability of the intervention, including protocol compliance, and to gain insights on the perceived experience (suitability of the intervention protocol, perceived benefits and/or barriers to adherence).

7.4 Methods

A pre-registered three-arm parallel randomized controlled trial design was chosen to compare two different behavioural sugar reduction techniques to lower preferred sweetness intensity in hot tea; (i) an immediate cessation of added sugar to tea protocol [IC], versus (ii) a gradual, progressive reduction of added sugar in tea (- 25% weekly) protocol [GR], relative to a wait-list control group. A wait list control group [WC] was chosen to act as the reference to determine the overall effectiveness of the intervention, and to allow wait-listed participants to take part in the intervention after completion of the study. A detailed overview of the study design is shown in **Figure 7.1.**

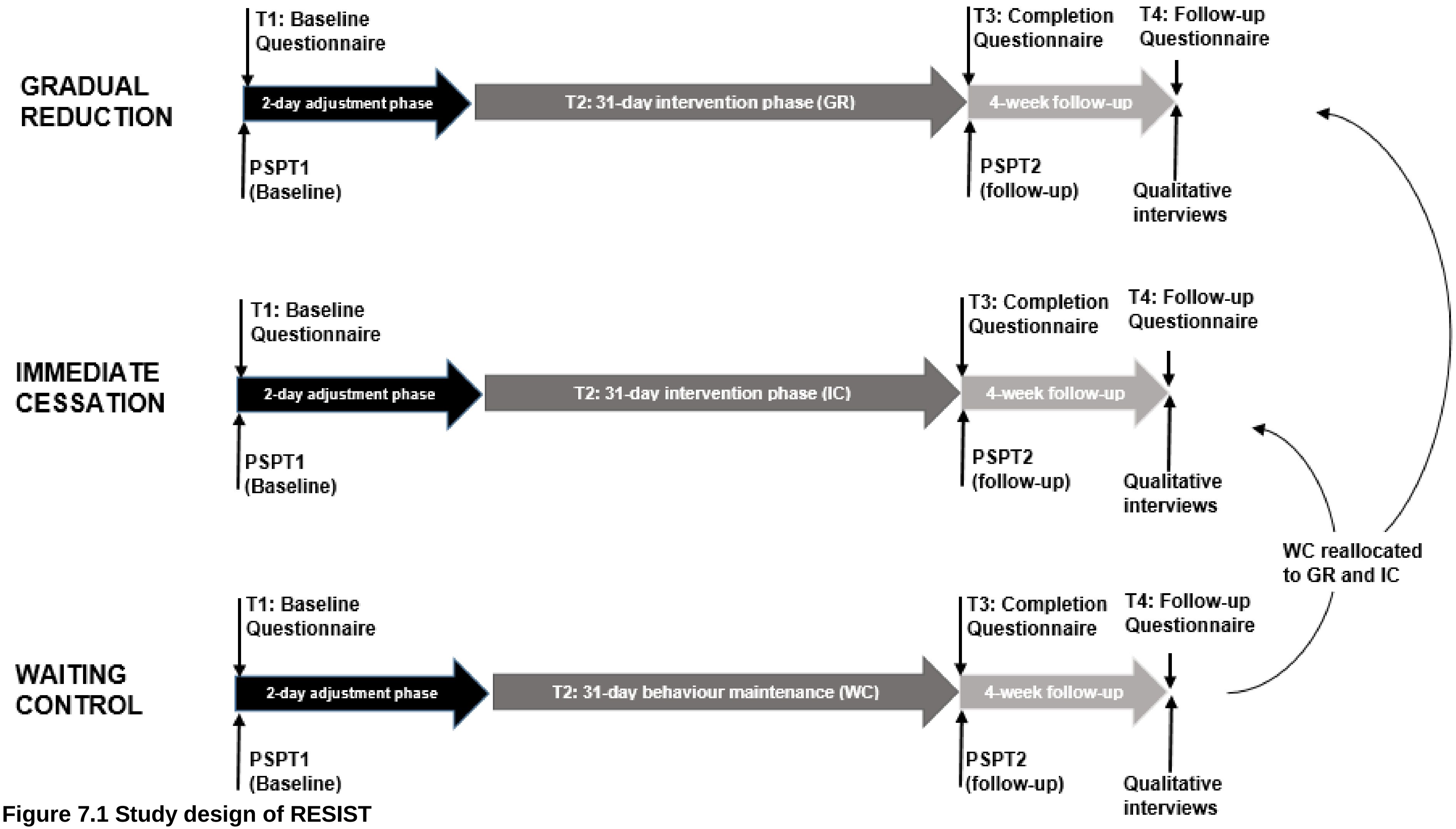


Figure 7.1 Study design of RESIST
Abbreviations: PSPT=Psychophysical sweetness preference test

7.4.1 Sample

1.1.1.1 Recruitment

Recruitment began in December 2016 to capitalize on the heightened interest and motivation to commit to healthy lifestyle changes at the start of the new year. Participants were predominantly university students (>18 years) at University College London (UCL), Kings College London (KCL) and Imperial College London. Recruitment occurred via various online communication channels; mainly via the weekly official university-wide student e-newsletter '*myUCL'* which is disseminated to 38,000 UCL students, or via targeted calls on social media platforms (Facebook and Twitter). An example of the featured recruitment message is shown in **Appendix F1.** Recruitment advertisements were also featured on the official *UCL Go!* smartphone application which UCL students use to access their timetable. Additionally, a recruitment e-mail was sent-out to a database of London-based undergraduate students (n=617) who had expressed an interest in participating in research studies led by the Department of Behavioural Science and Health.

7.4.1.1.1 Eligibility criteria

To participate in the pilot study, participants had to be ≥18 years of age and drink hot tea with added sugar daily. For this trial, participants were considered eligible if they reported occasional use of artificial sweeteners or honey, in addition to the daily use of sugar, to sweeten tea. Exclusion criteria for this study focused on participants who should not drastically alter their dietary habits without consulting a medical professional, e.g. during pregnancy, persons with a diagnosis of diabetes (type 1 or 2). Additionally, participants were excluded if they wouldn't be able to partake adequately due to unreliable access to a smartphone device (e.g. due to travelling at the time of the active intervention period, or not owning a smartphone).

7.4.1.2 Preliminary sample size calculation

Initial sample size calculations for the pilot trial were based on results reported from a previous dietary modification trial which assessed the change in salt taste perception after long-term reduction of dietary sodium intake (Bertino et al. 1982). Power analyses were based on sodium reduction data as it has been proposed that the biological mechanisms underlying the modification of sweetness and saltiness preferences may be related (Wise et al. 2016). Based on effect sizes seen in the salt taste perception trial, it was calculated that a minimum number of 12 participants per intervention arm would be needed to provide 80% power to detect a significant intervention effect (α=0.05) on rated pleasantness for sweetness of tea. Additionally, to account for drop-outs we assumed an attrition rate of 40% per trial arm. This is slightly above the cut-off point of 30%, the maximum acceptable level of attrition used by the CDC Prevention Research Team to identify robust and feasible behavioural interventions (Lyles et al. 2006). Based on our assumed attrition rate we therefore aimed to recruit a minimum of 17 participants per group (n_{total}=51).

7.4.1.3 Randomisation

Blocked randomisation, assuring equal size of the trial arms, was undertaken to account for known and unknown confounders. Before randomisation participants were invited to undergo a voluntary baseline psychophysical sweetness preference test (PSPT). The allocation ratio was set to 1:1:1 across each respective group, and randomisation was stratified by PSPT participation status. In that way, distribution of participants who had undergone the PSPT was balanced across the three intervention groups (Efird 2011).

Informed consent was obtained from all participants. The protocol for this pilot trial was approved by the Ethics Committee of University College London (Project ID *10005/001*) and was registered with the ISRCTN trial registry (ISRCTN ID number: 56753033) (**Appendix F2**).

7.4.2 Study procedure

All participants were required to complete a baseline questionnaire and sign a consent form prior to beginning of the intervention. Eligible subjects were randomised across the three study arms, each group receiving a tailored set of instructions for the course of the 31-day active intervention period.

Participants were randomly allocated to: (i) the ‘Immediate cessation’ [IC] group which was instructed to immediately cease the addition of any kind of sweetener to their tea throughout the duration of the entire intervention, (ii) a ‘Gradual reduction’ [GR] group which instructed participants to gradually reduce the amount of added sugar to their tea, and (iii) a waiting control group [WC].

In brief, the three arms of RESIST followed a similar timeline:

(1) <u>Recruitment:</u> Initiated at the end of December 2016. All volunteers were screened for eligibility.

(2) <u>Enrolment</u>: Eligible study participants received an e-mail with a link to the baseline questionnaire [*T1*]. Participants were given the opportunity to volunteer for an additional objective pre- and post-intervention sweetness preference measurement experiment, and for an additional post-intervention qualitative interview.

(3) Baseline psychophysical sweetness preference test (PSPT1)
(4) Randomisation stratified by PSPT participation status.
(5) Intervention pack dissemination: Intervention group-specific materials were e-mailed to all participants; Participants were instructed to download the accompanying RESIST phone application.
(6) Adjustment phase: The study began with a 2-day adjustment phase to standardize baseline measurements and to ensure that participants were familiar with the functionality of the RESIST phone app interface. Participants were instructed to maintain their usual eating and drinking behaviours but to start using the RESIST smartphone application on their phone.
(7) Active intervention period: The 31-day active intervention period during which the RESIST smart phone application was used to track participant's tea sweetness preference ratings and daily sugar intake from tea for the entire intervention [*T2*]
(8) A completion questionnaire [*T3*]
(9) Follow-up PSPT (PSPT2)
(10) Voluntary qualitative interviews
(11) Follow-up questionnaire four weeks' post-intervention completion *[T4*].

7.4.2.1 Intervention procedure and materials

After randomisation, individuals received an 'intervention pack' via e-mail, which contained tailored instructions relating to the specific condition to which they had been allocated. Copies of the test instruction booklets for each of the three conditions are shown in **Appendix F3 – F5.**

In the intervention booklet, subjects received their personal RESIST intervention schedule (GR, IC or WC), a small number of basic tips to reduce sugar in tea, a detailed guide on how to install the RESIST smartphone applications on their phone and a FAQ section summarizing key information regarding the study aim and usage of the app. The smartphone application used for RESIST was PACO (*The Personal Analytics Companion*), an open-source behavioural research platform which can be downloaded and used free of charge. In the participant information sheet, sent along with the RESIST booklet, participants received an anonymised Gmail address (e.g. 2017resist.01@gmail.com) to access the smartphone application once it had been activated on their personal device.

Tips provided in the intervention booklet were in-part based on habit theory to facilitate the creation of a new healthy habit or to break the existing habit of adding sugar in tea. For instance, one suggestion targeted a performance cue by encouraging participants to change up the arrangement of their usual tea making facilities by storing away any easily accessible sugar to prevent the automatic addition of sugar to their hot beverage (van't Riet et al. 2011). Other information contained in the booklet emphasized the importance of logging daily tea intake and daily sweetness preference scores on the RESIST smartphone application.

A set of plastic measuring spoons was sent to participants' term time home addresses, or was given to the participant directly if they had visited UCL to undergo the baseline psychophysical sweetness preference test. The measuring spoon set consisted of five separate scoops, each allowing the participant to accurately measure sugar in units of 1/8 of a tsp., ¼ of a tsp., ½ a tsp, 1 tsp, ½ tbsp. and 1 tbsp. The instruction leaflet sent along with the spoons is shown in **Appendix F6**.

Participants were sent a final study information sheet and asked to read the intervention instruction booklet before the start of the active sugar reduction phase (shown in **Appendix F7**). Subjects in the GR condition were instructed to start reducing added sugar (or the occasional use of honey or artificial sweeteners) in tea in accordance with their structured reduction protocol which mapped out how much and when the sweetness levels needed to be reduced. For example, in the first week, participants were asked to add only 75% of their usual sugar amount to tea. After a week at this level of sweetness, the next reduction phase in the protocol instructed participants to reduce this level to 50%, and so on until they reached 0% in the last week of the intervention. In contrast, the IC group were instructed to cut-out the addition of any sugar, honey or artificial sweeteners to sweeten tea from day 1 of the intervention protocol. Instead of being instructed to reduce the amount of sugar in their tea, subjects in the WC condition were encouraged to "be aware of any add-ins to their tea but to maintain their usual tea drinking habits". All participants (GR, IC, and WC) were instructed to track the number of tea cups they consumed each day, and the amount of sugar added to each cup by completing the daily questionnaire sent via the RESIST smartphone app. A daily record of any snacks or sweet treats consumed along with tea was also recorded to establish if sugar was compensated for in food.

Due to the nature of the intervention, it was not possible to blind intervention group allocation. As much as possible, participants randomised to the WC condition were kept naive with regards to their status as the control group. Subjects allocated to the WC group received a redacted version of the RESIST intervention booklet and the plastic measuring spoons. To encourage subjects in the waiting control group to actively track their drinking habits with the PACO app, they were told that they would progress to the active intervention stage if they tracked their tea drinking habits for four weeks. After completions of the follow-up questionnaire [T4], subjects in the WC group were sent the GR or IC sugar reduction protocol and were instructed to complete the protocol in their own time.

7.4.3 Strategy to ensure intervention fidelity

Several strategies were implemented to maximize intervention fidelity. A financial incentive (£10 Amazon voucher) was offered for participation, and these vouchers were awarded after completion of the intervention protocol. Participants who underwent both rounds of the psychophysical sweetness preference test and/or the qualitative interview, received an extra £10 Amazon voucher for each of these tasks.

At the half-way point of the intervention (day 15), a reminder and encouragement message was sent to all participants to keep motivation levels high. Specific reminder e-mails were sent to participants in the GR condition group on a weekly basis, reminding them that the next stage in their step-wise reduction intervention protocol was due to begin. Participants were also given the option to edit the time of their daily RESIST smartphone app notification. The default notification time was set to 9:30 pm every day. However, participants were provided with instructions on how to change this setting to select the most convenient time in their personal schedule.

7.4.4 Measures

Outcome and process measures were taken before randomisation (baseline [T1]), after completion of the 4-week intervention program [T3], and repeated 4 weeks post-intervention completion [T4]. Data were collected using a combination of online questionnaires (on the platform SurveyMonkey), daily smartphone notifications [T2], an optional in-person experimental psychophysical sweetness preference assessment, and an optional qualitative interview at intervention completion. Measures are described in the following section and copies of the questionnaires are provided in **Appendix F8-F10**.

7.4.4.1 Sociodemographic characteristics

Standard demographic information was reported at baseline, including age (in years), gender, and ethnicity. Participants were instructed to select their ethnicity from four possible categories: ‘Asian’, ‘Black’, ‘White’, ‘Mixed’, or ‘Other’. Information on current education status was collected by asking participants to select their current or highest achieved educational attainment from: ‘Undergraduate degree’, ‘Masters (or equivalent)’, or ‘PhD (or equivalent’). They also were instructed to report living arrangements during term time, which were categorized as follows: ‘Living at home with parents’, ‘Student halls’, ‘Own flat/house’, ‘Shared flat/house’, or ’Other’.

7.4.4.2 Anthropometric and other health behaviour characteristics

Weight (in kilograms) and height (in centimetres) were self-reported at the start of the intervention, and used to calculate initial BMI. Participants were also asked to rate their current health status, from Poor, Fair, Good, Very Good to Excellent. To assess current and past smoking status, participants were instructed to select from the following options: ‘Current daily smoker’, ‘Occasional smoker’, ‘Former daily smoker’, ‘Former occasional smoker’, or ‘Never smoker’. Furthermore, participants were also asked to indicate whether they currently followed or had followed a (restrictive) weight loss diet in the last year (yes/no). Baseline general liking for sweetness was assessed with the question ‘Would you say you have a “sweet tooth”?’, and response options included ‘Not at all’, ‘Not really’, ‘Neither agree/disagree’, ‘Somewhat’, or ‘Very much’.

7.4.4.3 Motivation and previous attempts to reduce sugar in tea

At baseline, participants were asked to rate how strongly they agreed with the statement ‘I want to reduce/eliminate added sugar in my tea’. Motivation was rated on a numerical scale, ranging from 1-100, with a higher score indicating higher motivation. Previous attempts to cut out sugar in tea were also recorded. Participants were asked to select a response from the following: ‘No, never’, ‘Yes, I tried but failed’, ‘Yes, I initially succeeded but then went back to my usual sweetness habits’, and ‘Yes, I successfully reduced the amount of sugar added to my tea and have maintained this reduced level’, or ‘Other’.

7.4.4.4 Intervention preference

To gauge initial intervention preferences, a question on intervention preferences was buried in the baseline questionnaire. Participants were asked ‘How you would like to reduce sugar in tea?’, with responses including ‘Abrupt/Immediate reduction’, ‘Gradual reduction’, or ‘No preference’. Participants were explicitly assured that indication of interest in one intervention approach would not influence actual intervention group allocation.

7.4.4.5 Measures of preliminary effectiveness of the intervention

The effectiveness of the intervention was assessed by examining three primary, and three secondary outcomes.

7.4.4.6 Primary outcome measures

There were three primary outcome measures: liking of sweetness in tea; intake of sugar from tea; and quit status. These were measured by self-reported questionnaires over three time points (T1, T3 and T4).

7.4.4.6.1 Liking of sweetness in tea

Initially in the baseline questionnaire, participants were required to report their current liking, sweetness level, and intake frequency of sweetened tea. Liking of sweetened tea was measured with the question 'How much do you like the sweetness level of your tea today?', with responses recorded on a 9-point Likert scale.

7.4.4.6.2 Intake of sugar from tea

Daily intake of sugar from tea was derived from total number of cups per day, and average amount of sugar added to each cup (recorded at baseline, at intervention completion, and at 4-week follow-up). Participants were required to indicate the average number of teaspoons of sugar they added to a cup of tea (~200 ml), from the following: 0.5 tsp, 1 tsp., 1.5 tsp., 2 tsp., 2.5 tsp., 3 tsp., 3.5 tsp., 4 tsp., 4.5 tsp., 5 tsp. and >5 tsp. The reported amount of sugar in tea was used to define the individual starting "dose" of amount of sugar in tea. To define daily overall sugar intake from tea, participants were required to report how many cups (1-5 cups) of tea they drink throughout various parts of an average day ('In the morning until midday', 'From midday until 6 pm', and 'After 6pm'). Total daily intake of added sugar from tea (in grams) was calculated by multiplying the number of cups of tea per day by the average number of teaspoons of sugar added to each cup. One teaspoon of sugar was defined as the equivalent of 4g of table sugar.

7.4.4.6.3 Quit status

Assignment of quit status ('Quitter', 'Reducer', and 'No change') was derived from the reported average amount of sugar added to each cup of tea at completion (T3) and follow-up (T4), relative to the amount reported at baseline (T1). Participants that no longer reported adding sugar to their tea at T3 (completion) or T4 (4-week follow up) were defined as 'quitters'. Participants that reported reductions in mean daily sugar intake of $\geq$2g/d were defined as 'reducers', and participants that reported a change of <2g per day for sugar added to tea were categorized as 'No change'.

7.4.4.7 Secondary outcome measures

There were three secondary outcomes: two compensatory behaviours (changing number of cups of tea consumed and using alternative sweeteners to sugar), and impact on general preference for sweetness.

7.4.4.7.1 Compensatory behaviours (number of cups of tea per day and change in tea add-ins)

Change in intake of number of number of cups of tea consumed per day was assessed in the completion (T3) and 4-week follow up questionnaire (T4).

To assess change in tea-add-ins, the question ‘Do you ever add artificial sweeteners to your tea, instead of sugar?’ was asked, and participants answered with ‘Yes’, ‘No’ or could elaborate in an ‘Other’ open text box. A further question asked about the habitual amount of milk or creamer added to tea (‘Never’, ‘Rarely’, ‘Occasionally’, ‘Always 1 Tbsp.’, ‘Always 2 Tbsp.’, ‘Always 3 Tbsp.’, About 50% tea/50%milk, or ‘Other’).

7.4.4.7.2 General preference for sweetness

A further secondary outcome was the objective psychophysical measure of preferred levels of sweetness which was collected at baseline (T1) and after completion of the intervention (T3). Testing was undertaken in a subset of study participants that had expressed an interest in this optional experiment in their baseline questionnaire. Measurements were collected using the Monell two-series, forced-choice, paired comparison tracking technique in which subjects are prompted to taste numerous pairs of sucrose solutions of different concentrations and to point to the solution they most prefer (Mennella & Bobowski 2016). A copy of the tracking grid used to assess response patterns is available in **Appendix F11**. Five suprathreshold sucrose solutions (**Table 7.1**; 3.0%, 6.0%, 12.00%, 24.0% and 36.0% weight/volume [wt/vol]) were prepared and presented in small disposable beakers, each containing identical amounts of the solution (~25 ml).

Table 7.4 Overview of the sucrose samples used in the forced-choice paired-comparison sweetness preference assessment protocol

Solution	Concentration (wt/vol)[1]	Ingredients
A	3.0%	3.0 g of sugar/ 97ml of water
B	6.0%	6.0 g of sugar/ 94 ml of water
C	12.00%	12.00 g of sugar/ 88.00ml of water
D	24.00%	24.00 g of sugar/ 76.00ml of water
E	36.00%	36.00 g of sugar/ 64.00ml of water

[1] *Abbreviation: wt=weight, vol=volume*

Participants were told to abstain from eating or drinking at least 1h prior to the assessment. The five samples were set out on a table but labelled with labels of alphabetic letters unknown to the participant. In accordance to a pre-set protocol, a first pair of solutions (Solution B at 6% wt/vol and Solution D at 24% wt/vol) was presented first. The participant was asked to taste each solution for five seconds, and then expectorate the sample into an opaque plastic cup. Between samples, participants were instructed to rinse their mouths with water before trying the second sucrose solution sample. Once both samples had been tried, subjects were asked to point at the sample which they liked better.

For the second set of paired comparisons, the preferred sample from the first tasting occasion was paired with the sample with the lower adjacent concentration. As in the first series of paired comparisons, the subject was prompted to try both solutions and to point to the beaker with the sample of the sweetness which they preferred. Crucial to this experimental procedure was that in Series 1 of this assessment, the weaker solution of the sample pair was always presented first. The paired comparisons were undertaken until the study participant chooses the same sample when presented with both a lower- and higher adjacent concentration sample, or when the participant selected the highest or lowest concentration sample as the preferred solution twice in a row. After a brief break of 3-5 minutes, the entire protocol is repeated. However, in contrast to Series 1 of the paired-comparisons where the lower concentration sample was presented first, in Series 2 the stronger concentration was presented first. Once again, the entire testing protocol was repeated until a preferred sample was determined using the same criteria as in Series 1. The overall most preferred level of sucrose was determined by calculating the geometric mean of the final concentration of the samples chosen in Series 1 and 2 of the tasting protocol (shown in **Equation** 1 below).

$$\sqrt{\frac{\%wt}{vol}[Series\ 1]} * \sqrt{\frac{\%wt}{vol}[Series\ 2]} = Sucrose\ intensity\ most\ preferred\ (\%\frac{wt}{vol})$$

Equation 1 Geometric mean of sucrose most preferred

The procedure has previously been tested and validated in populations of different ages and ethnicities as a quick and cheap method to determine a single preferred level of sweetness that avoids position bias (Mennella et al. 2011).

7.4.4.8 Appraisal of study procedure

7.4.4.8.1 Feasibility

Feasibility was assessed via multiple parameters to assess the potential for future successful implementation of the proposed intervention (Tickle-Degnen 2013). Parameters included recruitment rate (% of interested participants that successfully enrolled in the study), and retention rate (% of participants who completed T1, T2, T3 and T4). Resource and management feasibility of the protocol was tested by considering the suitability of the planned time frames to coordinate participants, to disseminate the intervention instructions, to send out the intervention packs, to test the functionality of the smartphone application, and to verify the reliability and accuracy of collected data (T1 - T4).

7.4.4.8.2 Acceptability analysis

Acceptability was tested by measurement of various parameters, including constructs in the Theoretical Framework of Acceptability of Healthcare interventions (TFA) detailed below (Sekhon, Cartwright & Jill J. Francis 2017).

7.4.4.8.2.1 Intervention compliance and engagement with protocol ("opportunity cost")

Compliance to the intervention was evaluated via the daily smartphone application data. Non-participation was assumed on days when no smartphone data ratings were received. Study participants were instructed to submit their app questionnaire daily and to explicitly report occasions on which they were non-compliant to their assigned taste modification protocol. Engagement with protocol was defined as the extent to which participants reported sticking to their allocated sugar instructions, and calculated as the proportion of days on which participants reported adhering to their assigned sugar reduction schedule (out of the 31 total days in the active intervention phase).

7.4.4.8.2.2 Convenience and suitability ("burden")

A series of statements on perceived effort of participating in the intervention, overall perceived ease of intervention protocol adherence, and completion of the smartphone application task were assessed at T3. Participants were asked to rate the extent to which they agreed with statements such as 'It was difficult to complete the daily smartphone task' or 'I found it difficult to drink my tea with a reduced amount of sugar in it'. Greater acceptability of the intervention protocol was indicated by a lower score on a 5-point Likert-scale (anchored by 'Strongly disagree' to 'Strongly agree').

7.4.4.8.2.3 Intervention benefits and barriers (“perceived effectiveness”)

At intervention completion (T3), perceived effectiveness of the intervention was assessed by asking participants to rate how much they agreed with the following statements: ‘RESIST reduced my preference for sweetness in tea’, ‘RESIST reduced my intake of sugar from tea’, and ‘Sweet food tastes sweeter or too sweet’. Participants rated their agreement with the statements on a 5-point Likert-scale from ‘Strongly disagree’ to ‘Strongly agree’, with higher scores indicating higher agreement. Open comment boxes at the end of the completion (T3) and follow-up questionnaire (T4) invited participants to elaborate on the quantitative measures of acceptability with personal comments on their RESIST experience.

7.4.4.9 Qualitative interviews

Qualitative interviews of 15 participants were undertaken to explore intervention acceptability, effectiveness and adherence in detail. The aim of the follow-up qualitative interviews was to explore which approach was most suitable, acceptable and effective for individuals who differed in their reasons for participation, sociodemographic characteristics and baseline tea drinking habits (Sekhon, Cartwright & Jill J Francis 2017). Selection of interviewees was designed to maximise the range of demographic characteristics, consumption habits, and level of compliance to obtain a wide overview of experiences of RESIST, and to achieve data saturation.

Interview structure and content was developed in collaboration with an MSc student (Sonam Verma; SV). SV conducted all interviews from March to April 2017. An interview schedule of 24 semi-structured questions was developed to ascertain six main aspects of the participant’s experience of the study (shown in **Appendix F12**). Questions captured participants’ main motivation to participate, their thoughts on the content of the study materials, the extent to which they benefitted from participation, the appropriateness of intervention delivery, the effectiveness of the study in decreasing their preference for sweetened tea, and any general comments to improve the study protocol. Interviews were recorded with a digital voice recorder, and transcribed by a professional transcription company (Devon Transcriptions Ltd).

The complete results of the qualitative evaluation of RESIST was submitted for the degree of MSc in Health Psychology (UCL) by SV in September 2017. The findings from the qualitative study are only briefly mentioned in the discussion section of the thesis and will be used to improve the design of RESIST in a fully-powered RCT.

7.4.5 Statistical analyses

A priori defined hypothesis testing in small pilot study data is acceptable if it is undertaken to inform future trial design (Thabane et al. 2010). In line with recommendations, initial analysis of RESIST pilot data has been undertaken for this purpose and is clearly indicated as such in the results section. Moreover, reporting of preliminary quantitative results has focused on the interpretation of confidence intervals of obtained estimates (rather than precision of estimates) of initial effect sizes (Lancaster 2015).

In line with the CONSORT guidelines for RCTs, baseline characteristics were not formally assessed for mean group differences of sociodemographic or other characteristics as intervention condition allocation was determined by blocked randomisation (Moher et al. 2010). Analysis was by intention-to-treat, meaning that data from all participants was analysed as per original treatment assignment, irrespective of compliance to the assigned intervention condition. The dataset was inspected to ensure it met the necessary assumptions for analysis of variance (ANOVA) models.

7.4.5.1 Analysis of primary and secondary outcomes of the intervention

7.4.5.2 Continuous outcomes

Two-factor mixed-design ANOVAs were used to assess the effect of the intervention on changes from baseline (T1) to completion (T3) and follow up (T4) in continuous outcomes: mean preference ratings for tea; daily sugar intake from tea; daily number of cups of tea consumed; and general sweetness preference (measured by the psychophysical test). There was one between-group factor (condition), with three levels (GR, IC, WC) and one within-subjects factor (time) with three levels (T1, T3, T4). For the analyses conducted on general sweetness preferences, the within-subjects factor (time) had two levels (T1 and T3).The effect of the interventions on change from baseline to completion and follow up was tested through an interaction term between group and time (a significant interaction would indicate that change from baseline to completion and follow up varied by group). Posthoc pairwise comparisons of outcomes across the three groups adjusting for basleine were planned at T3 and T4 if the interaction term was significant.

To justify use of ANOVA models to test for the significance of differences between the means of three or more independent groups, the following assumptions were tested and met:

[1] Normality of the residuals: This was assessed in QQ plots of the standardized residuals compared to the distribution of standardized residuals of a normal distribution.

[2] Homogeneity of variance: The variance of all data points of the dependent variable needs to be constant for each group. This was checked using Levene's test.

[3] Independence of the observations: All observations need to be independent i.e. there can be no relationship between the observations in each group or between the groups themselves.

For repeated-measures ANOVA an additional assumption needs to be satisfied:

[4] Sphericity: Variances of the differences between all possible pairs of within-subject data points must be equal. This can be formally examined using Mauchley's test. If the sphericity assumption was violated (Mauchley's $p \leq 0.05$), then the Green-Geisser correction was used to reduce the risk of a type I error.

Independent groups t-tests were used to analyse acceptability indicator data at intervention completion and 4-week follow-up separately.

7.4.5.3 Categorical outcomes

Chi-square tests were undertaken to test for differences in proportions of quit status and change in add-ins to tea (creamer and non-nutritive sweeteners) between the three conditions at completion, and at 4-week follow-up. Subsequent chi square tests were used to test for differences between proportions of quit status between the intervention conditions (GR and IC combined) and the control group.

7.4.6 Power and statistical significance for tests of preliminary effectiveness of intervention

For all tests the alpha level was set at 0.05, and statistical analyses were conducted in SPSS (Version 22.0; SPSS Inc.) For ANOVAs the effect size was estimated using partial Eta squared (η_p^2) which equates to the proportion of the variance explained by the independent variable in question. For paired t-tests, the effect size was estimated with Cohen's d. For unpaired t-tests, effect size was estimated using Hedges' g, which takes into account unequal sample sizes.

7.4.6.1 Estimating sample size for a fully-powered randomised controlled trial

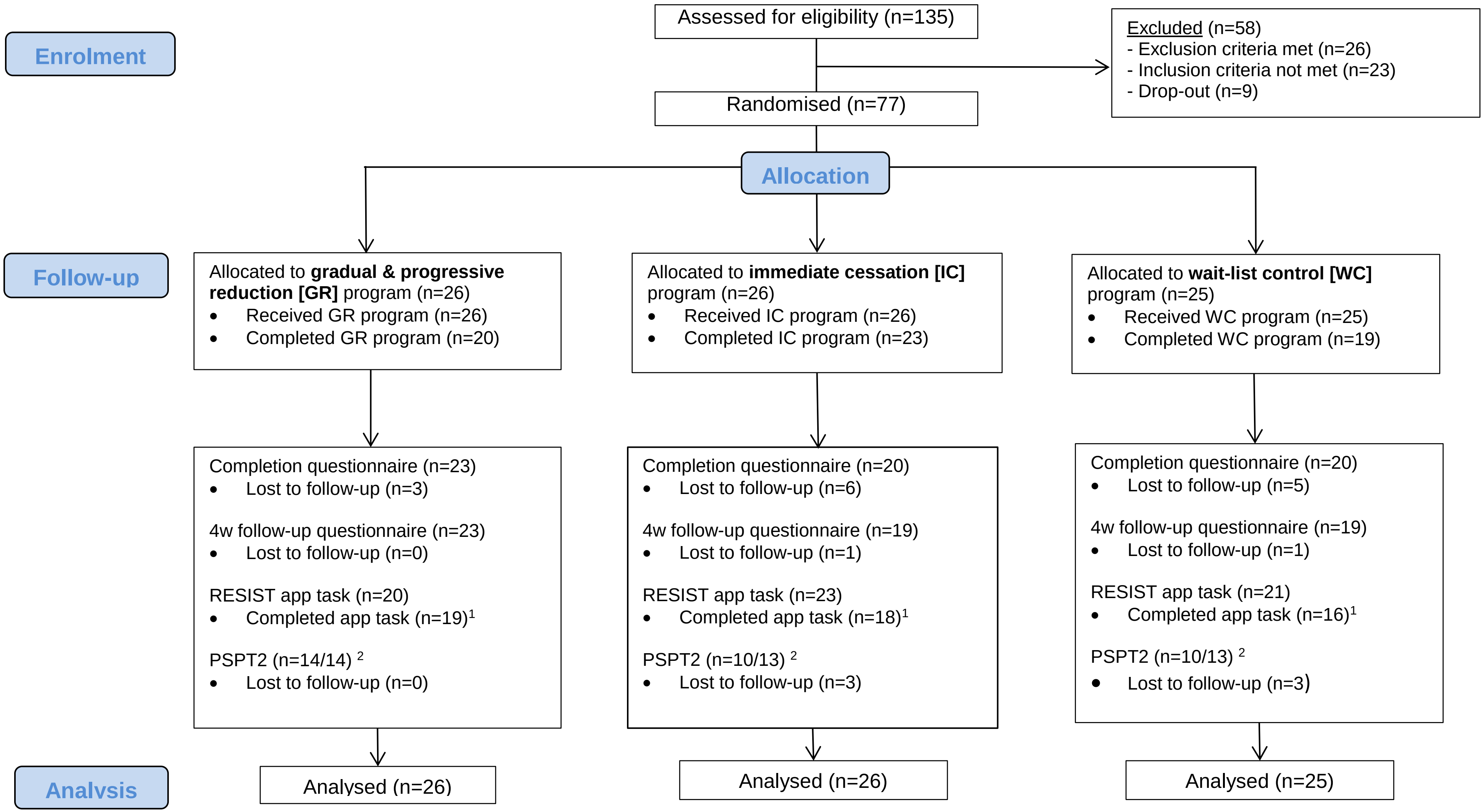
The required sample size for a fully-powered randomised controlled trial was estimated based on the effect sizes observed in the analyses of the preliminary effectiveness of the intervention, based on 80% power and an alpha level of .05. Power analysis was conducted in G*Power (Version 3.1.9.2; Softpedia).

7.5 Results

7.5.1 Summary statistics

Recruitment resulted in 177 students registering their email addresses via an online database as an initial indication of interest. Invitations to formally enrol into RESIST were disseminated via e-mail, and 135 students (76.27%) proceeded to sign-up and complete the baseline questionnaire. Of the 135 students that completed the baseline questionnaire, 23 were excluded as they did not report drinking sugar-sweetened tea daily. A further 26 volunteers were excluded as they did not have reliable access to a smartphone device, or would be travelling at the time of the intervention (March – April 2017). A small number of individuals (n=9) started but did not complete the online baseline questionnaires. Overall, a total sample size of n=77 was randomised across the three intervention conditions. Blocked randomisation resulted in three equally sized intervention groups (n=26 in GR, n=26 in IC, and n=25 in WC). The flow of participants through RESIST is summarized in **Figure 7.2**.

Figure 7.2 CONSORT flow chart of participants1



[1]Completion of app survey task was defined as submitting data via the RESIST smartphone application ≥10 day of the 31 days of the active intervention phase
[2]*Abbreviation: PSPT= Psychophysical sweetness preference test*

Overall, most of the sample was female (80.5%), and mean participant age was 23 years (SD=5.6). Approximately half of the participants were of Asian ethnicity (45.5%), followed by participants of White (39%), Mixed (9%) or Black (5.2%) ethnic background. Most were undergraduate students (71.5%), and reported living in a shared house or flat (44.2%) during term time. The sample had a lean mean BMI (22.3 kg/m^2 [SD=3.5]) and 67.6% reported being in good, very good or excellent general health; 74% were non-smokers, representative of national smoking prevalence statistics among 18-24 year olds in the UK (PHE 2017). Motivation to reduce and quit sugar was high at 67.5/100 (SD=30.3). Two-thirds of the participants (67.6%) had previously attempted to reduce sugar in their tea. When asked whether participants had a preferred method of reducing the amount of sugar in tea, 57.1% of the sample indicated a preference for a gradual, progressive reduction strategy. A detailed break-down of baseline socio-demographic characteristics of the sample is shown in **Table 7.2.**

Baseline tea intake was 2.72 (SD=1.62) cups of tea per day. On average, participants added 1.46 (SD=0.65) teaspoons of sugar to each cup of tea, equivalent to 5.84g of sugar per cup. The average participant therefore had a mean daily intake of 14.59g (SD=8.69) of free sugar from tea at baseline, roughly equivalent to 50% of an adults total daily recommend sugar intake (World Health Organization 2015). Preference scores for tea prepared in the participants' usual and preferred manner were high, with a mean preference score of 6.73 (SD=1.67) out of a possible maximum of 9. Most participants (n=52; 81.3%) added milk or creamer to their tea. Only a small proportion of participants (n=12; 18.8%) reported occasional use of a non-nutritive sweetener (e.g. Stevia, Canderel, Sweetex) to sweeten their tea.

Table 7.2 Baseline characteristics of participants by RESIST condition

Characteristic	**Gradual Reduction** (*n=26*)	**Immediate cessation** (*n=26*)	**Wait-list control** (*n=25*)	**Total** (*n=77*)
Age [mean (SD)]	23.0 (4.7)	22.6 (4.8)	23.3 (7.2)	23.0 (5.6)
Sex [*n* (%)]				
M	6 (23%)	4 (15.4%)	5 (20.0%)	15 (19.5%)
F	20 (77%)	22 (84.6%)	20 (80.0%)	62 (80.5%)
Race [*n* (%)][1]				
White	12 (46.2%)	8 (30.8%)	10 (40.0%)	30 (39.0%)
Black	1 (3.8%)	2 (7.7%)	1 (4.0%)	4 (5.2%)
Asian	10 (38.5%)	14 (53.8%)	11 (44.0%)	35 (45.5%)
Mixed	3 (11.5%)	2 (7.7%)	2 (8.0%)	7 (9.0%)
Other	0 (0.0%)	0 (0.0%)	1 (4.0%)	1 (1.3%)
Current education status [*n* (%)][1]				
Undergraduate	19 (73%)	17 (65.4%)	19 (76.0%)	55 (71.5%)
Masters (or equivalent)	3 (11.5%)	8 (30.8%)	4 (16.0%)	15 (19.5%)
PhD (or equivalent)	4 (15.5%)	1 (3.8%)	2 (8.0%)	7 (9.0%)
Term time residence [*n* (%)][1]				
Living at home with parents	3 (11.5%)	6 (23.0%)	4 (16.0%)	13 (16.9%)
Student halls	5 (19.2%)	8 (30.8%)	10 (40.0%)	23 (29.8%)
Own flat/house	0 (0.0%)	2 (7.7%)	3 (12.0%)	5 (6.5%)
Shared flat/house	16 (61.5%)	10 (38.5%)	8 (32.0%)	34 (44.2%)
Other	2 (7.7%)	0 (0.0%)	0 (0.0%)	2 (2.6%)
BMI[2] [mean (SD)]	22.6 (3.6)	21.4 (2.7)	22.8 (4.2)	22.3 (3.5)
General health [*n* (%)][1]				
Poor	0 (0.0%)	0 (0%)	0 (0%)	0 (0.0%)
Fair	7 (26.9%)	10 (38.5%)	8 (32%)	25 (32.5%)
Good	15 (57.7%)	9 (34.6%)	14 (56%)	38 (49.4%)
Very good	3 (11.5%)	6 (23%)	3 (12%)	12 (15.6%)
Excellent	1 (3.8%)	1 (3.8%)	0 (0%)	2 (2.6%)
Smoking status [*n* (%)][1]				
Current daily smoker	1 (3.8%)	0 (0.0%)	2 (8.0%)	3 (3.9%)
Occasional smoker	3 (11.5%)	3 (11.5%)	2 (8.0%)	8 (10.4%)
Former daily smoker	0 (0.0%)	1 (3.8%)	1 (4.0%)	2 (2.6%)
Former occasional smoker	4 (15.5%)	2 (7.7%)	1 (4.0%)	7 (9.0%)
Never smoker	18 (69.2%)	20 (76.9%)	19 (76.0%)	57 (74.0%)
Motivation to quit sugar in tea[2] [mean (SD)]	64.6 (32.1)	63.1 (30.7)	75.0 (27.68)	67.5 (30.3)
Age of tea drinking initiation [mean (SD)]	11.56 (5.1)	11.65 (4.9)	11.64 (4.6)	11.62 (4.8)
Previous efforts to quit sugar in tea [*n* %)][1]				
No	7 (26.9%)	10 (38.5%)	8 (32.0%)	25 (32.5%)
Yes, but unsuccessful	11 (42.3%)	5 (19.2%)	5 (20.0%)	21 (27.3%)
Yes, initially successful but went back to normal	3 (11.5%)	4 (15.5%)	3 (12.0%)	10 (13.0%)
Yes, and have maintained success	5 (19.2%)	7 (26.9%)	9 (36.0%)	21 (27.3%)
Intervention preference [*n* (%)][1]				
IC[2]	3 (11.5%)	3 (11.5%)	5 (20.0%)	11 (14.2%)
GR[2]	16 (61.5%)	12 (46.2%)	16 (64.0%)	44 (57.1%)
No preference	7 (26.9%)	11 (42.3%)	4 (16.0%)	22 (28.6%)

[1] Percentages do not always add up to 100% due to rounding
[2] Motivation was measured on a visual analogue scale, anchored by 0 'no motivation' to 100 'highly motivated
[3] *Abbreviation: BMI=Body mass index*

7.5.2 Primary outcomes (liking, sugar intake, and quit status)

Analysis of primary outcome measures was restricted to participants who completed the daily RESIST smartphone application task (n=64). Despite participants in the control group being instructed to maintain their usual eating and drinking behaviours (specifically with regards to the add-ins in tea), subjects were not blinded to the aim of the study and highly motivated to change their behaviour. Results indicated that a large proportion of subjects in the WC group attempted to reduce the amount of sugar on their own, without receiving the full RESIST intervention materials.

7.5.2.1 Change in liking of tea

Overall, the means did not indicate much difference in change in liking of tea from baseline to completion and follow up for any of the three groups. In keeping with this observation, the two-way mixed ANOVA (**Table 7.3**) showed there was not a significant main effect of time (F(2,55)=1.30 p=.28, η_p^2=.02) indicating that overall there was no change in liking of tea from baseline to completion to follow-up. And most importantly, nor was there a significant interaction effect between group and time, meaning that liking of tea over time was the same across the three groups ((F(2,55)=1.303, p=.06, η_p^2=.10; T1=6.73; T3=6.30; T4=6.71); suggesting that the intervention did not detrimentally affect liking of tea. Results are graphically shown in **Figure 7.4**.

7.5.2.2 Changes in intake of sugar from tea

The means suggested that there was a reduction in sugar intake from baseline to completion and follow up for all three groups, whether this was measured by teaspoons or grams of sugar. This was supported by significant main effects of time for both teaspoons of sugar (F(2,55)=21.81, p<.001, η_p^2=.29), and grams of sugar (F(2,55)=4.37, p=.03, η_p^2=.07), indicating that overall all participants had reduced the amount of sugar they took in their tea at completion and follow-up compared to baseline. Mean change in sugar intake from tea at completion and follow-up is shown in **Figure 7.5**. However, there was not a significant time by group interaction effect for sugar intake in either grams, ((F(2,55)=2.30, p=.06, η_p^2=.10; T1=6.73; T3=6.30; T4=6.71), or number of teaspoons ((F(2,55)=1.88, p=.46, η_p^2=.05); indicating that the extent of change over time was the same across the three groups and that there was not a significant effect of the intervention. Results are summarized in **Table 7.3.**

7.5.2.3 Quit status

Figure 7.6 presents an overview of the proportion of participants who successfully quit sugar at completion and at 4-week follow-up of RESIST. At intervention completion, eight participants each in the GR (42.1%) and IC (36.4%) groups had quit sugar in tea, compared to only one WC participant (5.6%). Quit success (% of quitters) was significantly higher in the two intervention groups combined compared to controls ($\chi^2(2) = 7.42$; p=0.03), but did not differ between intervention groups ($\chi^2(2) = 0.80$, p=0.67). Effect maintenance of the intervention was still apparent four weeks after the intervention, with six participants of the GR group (33.3%), and 10 participants in the IC group (45.5%), maintaining a 'quitter' status. Overall, there was no significant time by group interaction effect for quit success (p=.44, η_p^2=.31), and no evidence for a difference in effect maintenance between the intervention conditions at 4-week follow-up ($\chi^2(2)$=0.87, p=0.65).

Table 7.3 Means and mean changes in study outcomes at baseline, completion and 4-week follow-up

		Time						Within-subjects effect[1]		Between-group effect[1]		Interaction (time*group)	
Characteristic		n_{T1}	**Baseline**	n_{T3}	**Completion**	n_{T4}	**Follow-up**	**p**	η_p^{2}*	**p**	η_p^{2}*	**p**	η_p^{2}*
Primary outcomes													
Liking of sweetness in tea[4] [mean (SD)]													
	GR[6]	20	6.90 (1.83)	20	6.10 (2.67)	18	6.83 (2.15)						
	IC[6]	23	6.96 (1.43)	22	5.64 (1.50)	22	6.27 (2.00)						
	WC[6]	21	6.33 (1.77)	19	7.26 (1.76)	19	7.11 (1.52)						
	Total	64	6.73 (1.67)	61	6.30 (2.11)	59	6.71 (1.91)	.28	.02	.52	.02	.06	.10
Daily sugar intake (g) from tea [mean (SD)]													
	GR	20	**15.6 (11.18)**	**19**	**6.00 (6.24)**	**18**	**8.44 (8.57)**						
	IC	23	**13.82 (6.77)**	**22**	**9.41 (7.31)**	**22**	**6.50 (5.32)**						
	WC	21	**14.47 (8.20)**	**18**	**16.72 (14.63)**	**19**	**15.0 (16.00)**						
	Total	64	**14.59 (8.69)**	**59**	**10.54 (10.64)**	**59**	**9.83 (11.17)**	**.03**	**.07**	**.04**	**.11**	.09	.08
# tsps. of sugar/cup[3] [mean (SD)]													
	GR	20	**15.6 (11.18)**	**19**	**6.00 (6.24)**	**18**	**8.44 (8.57)**						
	IC	23	**13.82 (6.77)**	**22**	**9.41 (7.31)**	**22**	**6.50 (5.32)**						
	WC	21	**14.47 (8.20)**	**18**	**16.72 (14.63)**	**19**	**15.0 (16.00)**						
	Total	64	**14.59 (8.69)**	**59**	**10.54 (10.64)**	**59**	**9.83 (11.17)**	**.03**	**.07**	**.04**	**.11**	.09	.08
Secondary outcomes													
# cups of tea/day[3] [mean (SD)]													
	GR	20	6.90 (1.83)	20	6.10 (2.67)	18	6.83 (2.15)						
	IC	23	6.96 (1.43)	22	5.64 (1.50)	22	6.27 (2.00)						
	WC	21	6.33 (1.77)	19	7.26 (1.76)	19	7.11 (1.52)						
	Total	64	6.73 (1.67)	61	6.30 (2.11)	59	6.71 (1.91)	.28	.02	.52	.02	.06	.10
Addition of milk/creamer to tea [n (%)]												χ^2	P[5]
	GR	20	15 (75.0)	20	17 (85.0)	18	16 (88.9)						
	IC	23	19 (82.6)	22	19 (86.4)	22	16 (72.7)						
	WC	21	18 (85.7)	19	15 (78.9)	19	16 (84.2)						
	Total	64	52 (81.3)	61	51 (83.6)	59	48 (81.4)					11.20	.67
Occasional use of NNSs[3] in tea [n (%)]													
	GR	20	3 (15.0)	20	3 (15.0)	18	3 (16.7)						
	IC	23	5 (21.7)	22	5 (22.7)	22	3 (13.6)						
	WC	21	4 (19.0)	19	1 (5.3)	19	2 (10.5)						
	Total	64	12 (18.8)	61	9 (14.8)	59	8 (13.6)					.30	.86

[1] Two-factor mixed-design ANOVA
[2] An average cup of tea was defined as ~200ml.
[3] A teaspoon of sugar was defined as 4 grams of sugar, equivalent to the amount in sugar sachets and sticks commonly available in commercial food and drink outlets.
[4] Liking of tea was measured on a 9-point Likert scale, with a higher score indicative of higher liking.
[5] P-value for Chi-square test for goodness of fit
* Partial Eta squared (η_p^2) is a measure of effect size ranging from 0-1. Values are interpreted as >0.01 = small effect, >0.06= medium effect, and > 0.14=large effect
[6] *Abbreviations: NNSs= Non-nutritive sweeteners; GR= Gradual and progressive sugar reduction group; IC= Immediate cessation of sugar group; WC= Wait-list control group;* η_p^2*= Partial eta squared*

Figure 7.4 Mean liking of tea (±SEM) in the three RESIST groups at intervention completion and follow-up

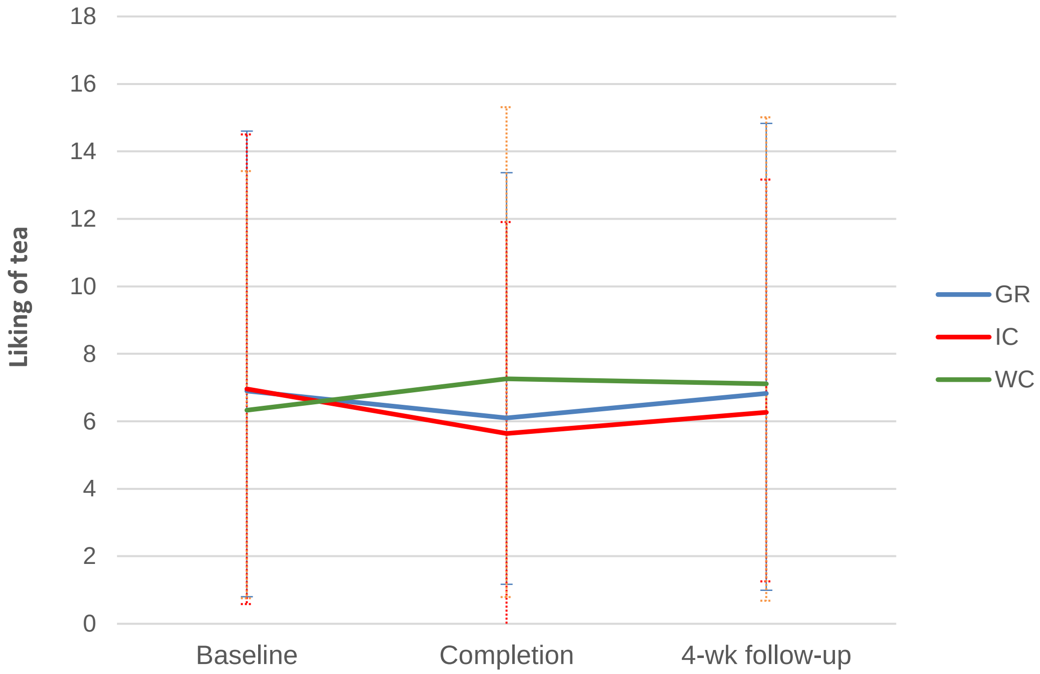
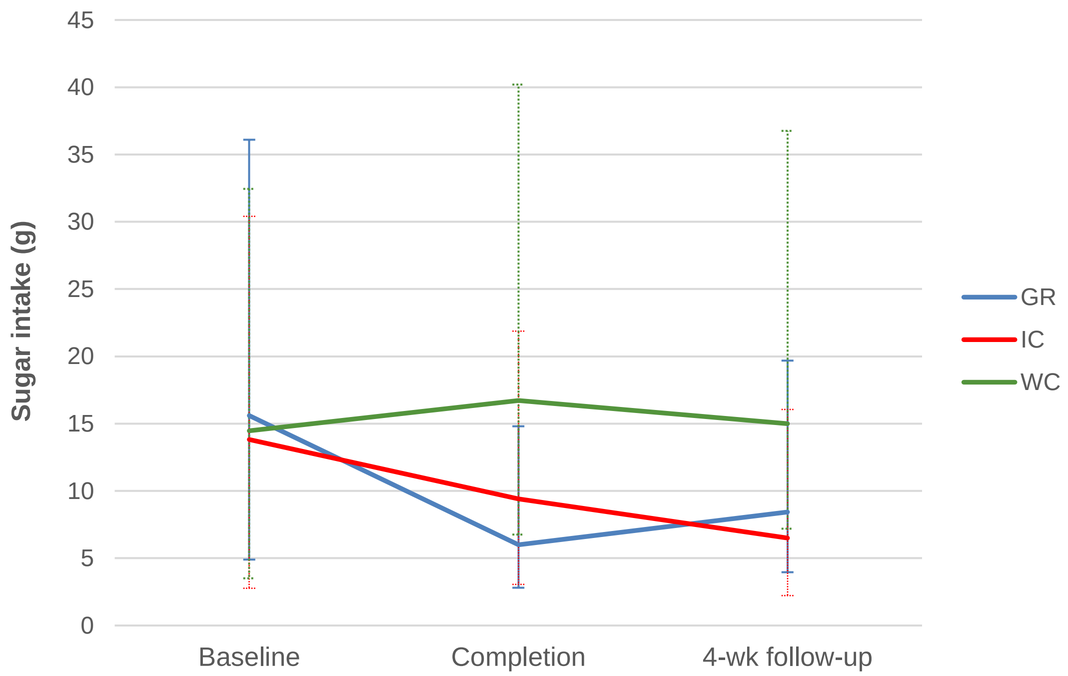
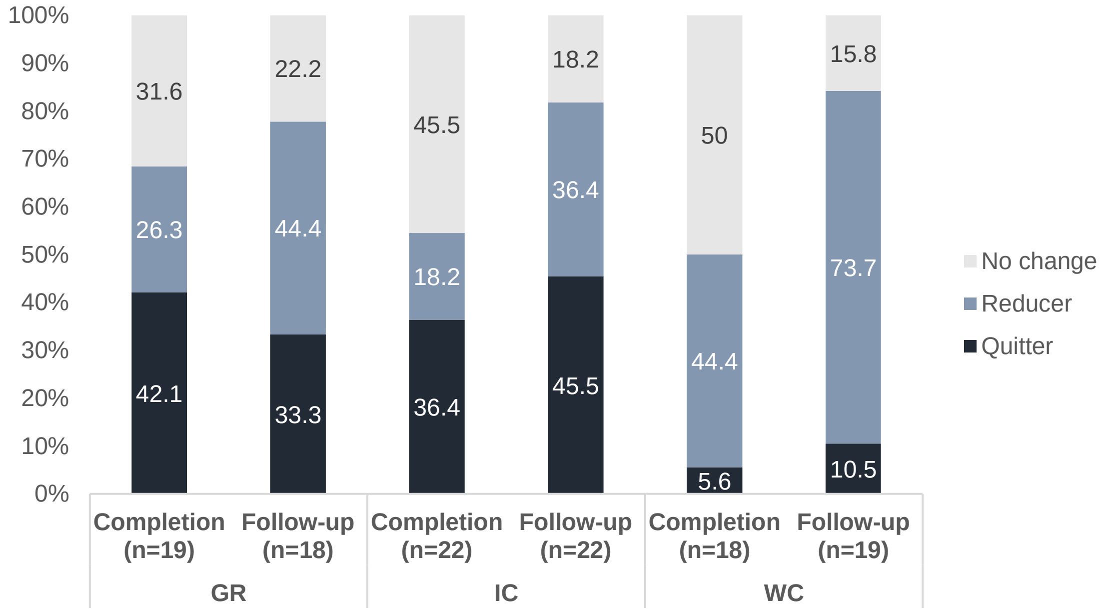


Figure 7.5 Mean daily intake of sugar from tea (±SEM) in the three RESIST groups at intervention completion and follow-up



Abbreviations: GR: Gradual and progressive sugar reduction group; IC: Immediate cessation of sugar group; WC: Wait-list control group

Figure 7.6 Quit status (%) by RESIST condition at completion and follow-up



Abbreviations: GR=Gradual sugar reduction group; IC=Immediate cessation sugar reduction group; WC= Wait-list control group.

7.5.3 Secondary outcomes

7.5.3.1 Compensatory behaviours

7.5.3.1.1 Change in intake of number of cups of tea

The means indicated that there was no clear pattern in change in the number of cups of tea consumed per day from baseline to completion to follow up for any of the three groups (**Table 7.3**). This finding was supported by a non-significant main effect of time ($F(2,55)=0.32$, $p=.64$, $\eta_p^2=.01$), indicating no meaningful change in the number of cups of tea consumed from baseline to completion to follow up for all participants. In addition, there was no significant time by group interaction for daily number of cups of tea ($F(2,55)=1.88$, $p=.46$, $\eta_p^2=.07$), indicating that change in the number of cups consumed per day was the same across the three groups from baseline to completion to follow up, suggesting that the intervention did not result in reduced tea consumption.

7.5.3.1.2 Change in tea add-ins

The percentage of participants who added milk or creamer to their tea was relatively constant over the study period (T1:81.3%, T2:83.6%, T4:81.4%), and there was no evidence that participants changed the amount of milk added to their daily tea to compensate for less sweetness during the intervention ($\chi^2(2)=11.20$; p=0.67). Usage frequency of non-nutritive sweeteners also did not change significantly ($\chi^2(2)=.30$; p=0.86). Proportions of users remained low throughout the intervention and 4-week follow-up phase (T1:18.8%, T2:14.8%, T4:3.6%). Findings on the tea add-ins are summarized in **Table 7.3**.

7.5.3.2 Preferred psychophysical level of sweetness

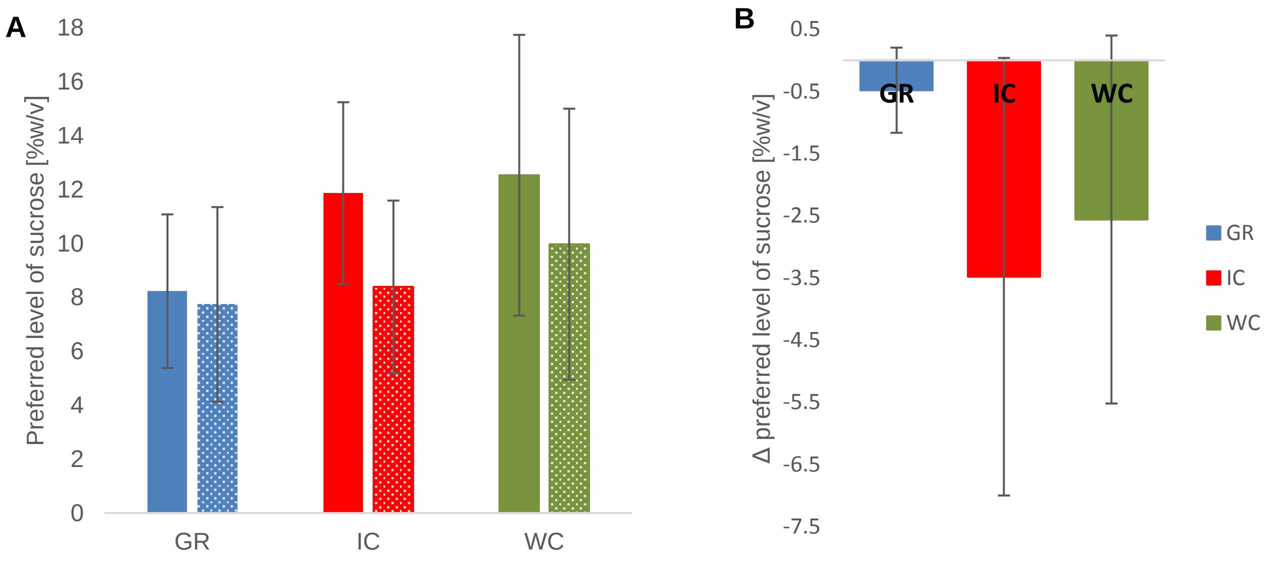
Among the 40 participants recruited to undergo the experimental preferred sweetness intensity psychophysical assessment, 34 (85%) returned for the second visit after intervention completion. Only data from subjects who completed both the pre- and post-intervention testing session are presented, with results from the psychophysical sweetness preference test summarised in **Table 7.4**. At the first visit, the mean preferred sucrose intensity level was 10.56 %wt/vol (median:8.49%wt/vol; range:3-29.39%wt/vol). Overall, there was a significant main effect of time ($F(1,31)=6.524$, p=.02, $\eta_p^2=.17$), indicating that overall there was a decrease in preferred intensity of sucrose from baseline to completion. The subgroup means suggested that this was larger for the IC (-3.48; SD=5.63) and WC (-2.56; SD=4.77) than for the GR group (-0.48; SD=4.42). However, there was no significant interaction effect between time and group, indicating that change over time did not differ significantly by group ($F(2,31)=1.193$, p=0.32), as shown in **Figure 7.7**.

Table 7.4 Psychophysical sweetness preference measures by RESIST condition

Preferred sweetness measures (SD)	Overall	Intervention condition			Within-subjects effect[1]		Between-group effect[1]		time*condition[1]	
	(*n*=34)	GR (*n*=14)	IC (*n*=10)	WC (*n*=10)	p-value	η_p^2	p-value	η_p^2	p-interaction	η_p^2
Baseline sucrose most preferred, geometric mean *[%wt/v]*	10.56 (6.55)	8.23 (5.44)	11.87 (5.43)	12.53 (8.40)						
Follow-up sucrose most preferred, geometric mean *[%wt/v]*	8.59 (6.71)	7.74 (6.90)	8.39 (5.17)	9.97 (8.15)						
Δ in sucrose most preferred *[%wt/v]*[3]	-1.97 (4.92)	-0.48 (4.42)	-3.48 (5.63)	-2.56 (4.77)	.02	.17	.43	.05	.32	.07

[1] Assessed using two-way mixed ANOVA
Abbreviations: GR=Gradual reduction group; IC=Immediate cessation group; WC=Waitlist control group

Figure 7.7 Change in psychophysical sucrose preference scores after RESIST by condition



A: Mean baseline and post-RESIST preferred level of sucrose. Baseline data is shown as the opaque bar graphs; post-intervention preferences scores are shown in the patterned bar graphs.
B: Change in preferred level of sucrose after RESIST. Change score calculated by subtraction of the post-intervention preference scores from baseline measurement.

Error bars indicate the SD of the mean values. Sample sizes for each group were as follows: GR: n=14; IC: n=10; WC: n=10.
Abbreviations: GR=Gradual reduction group; IC=Immediate cessation reduction group; WC=Wait-list control group

7.5.4 Feasibility analysis

Interest in the intervention was moderate, with 177 subjects registering preliminary interest on an online database. Of these, 77 participants were enrolled into the trial, corresponding to a recruitment rate of 57%. Retention rate for the completion questionnaire was 83.1%, with 64 out of the 77 participants completing both the smartphone task and the completion questionnaire (T3). At 4-week follow-up, retention rate was 76.6% (n=59/77). Questionnaire packs were sent to participants two weeks prior to intervention beginning (including the plastic measurement spoons), and all but one of these were received on time to start the active intervention period. Coordination of participants throughout the course of the intervention via e-mail (e.g. intervention booklet dissemination, reminders to complete questionnaires etc.) was prompted, and this was manageable for one researcher. All participants (100%) could successfully install the RESIST smartphone application on their devices. During the adjustment phase (pre-T2), 14 (21%) participants reported minor technical problems regarding the submission of their daily app surveys but these issues were swiftly dealt with via e-mail. The PACO desktop database management platform operated as intended and reliably tracked participants' daily smartphone data entries.

7.5.5 Acceptability analysis

As part of intervention follow-up [T3], a section to ascertain intervention acceptability was included. At intervention completion, n=23 (88.5%) of the GR group, n=20 (76.9%) of the IC group and n=20 (80%) of the WC group completed the questionnaire (n=63 in total). Maintenance of perceived benefits was measured in the follow-up questionnaire (T4), completed by n=23 (88.5%) in the GR group, n=19 (73.1%) in the IC group, and n=19 (76%) in the WC group (n=61 in total).

In general, participants in the both intervention groups (GR and IC) felt that participation in the study was easy and straightforward. A summary of the results is shown in **Figure 7.7C**. No significant differences were reported between the GR and IC groups for the difficulty in completing the entire 2-month period of the RESIST study (mean GR 1.90 vs. mean IC: 1.87; p=.91), the adherence to the respective sugar reduction protocol (mean GR: 2.35 vs. mean IC: 2.43; p=0.80), or for drinking tea with a reduced amount or complete elimination of sugar (mean GR: 2.55 vs. mean IC: 2.43; p=.69). The full results are tabulated in **Appendix F13a and F13b.**

7.5.5.1 Intervention compliance and engagement with protocol

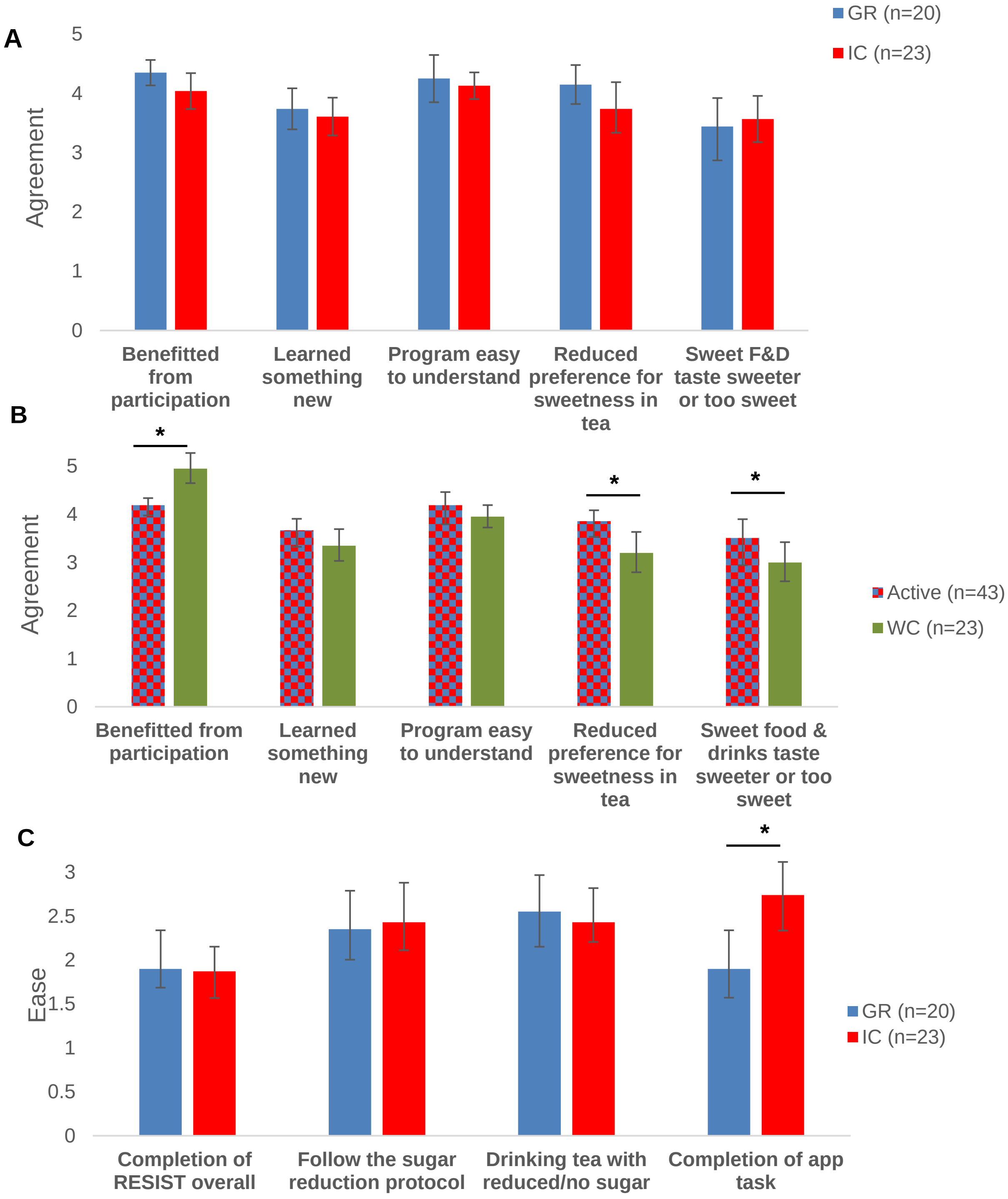
Engagement with the intervention was moderate, with participants following their allocated daily protocol on an average of 16.4 (SD=8.7) days. Mean values were nonsignificantly lower (t(41)=1.40, p=0.17) in the IC condition (n=23; 14.7 days [SD=9.5]) compared to mean engagement rate in the GR group (n=20; 18.5 [SD=7.4]).

7.5.6 Intervention benefits and barriers affecting perceived effectiveness

Selected outcomes are presented in **Figure 7.7A and 7.7B.** Overall, participants benefitted greatly from the intervention with a mean agreement score of 4.42/5 (SD=0.91) at completion and 4.48/5 (SD=0.95) at follow-up. No significant differences were seen between the GR and IC scores at either completion (mean =0.31, t(41)=-1.630; p=0.11) or follow-up (mean =0.13, t(39)=0.66, p=0.51).

Participants in the intervention groups combined reported greater agreement that they had experienced substantial reduction in the liking for sweetness of tea compared to the control group, and this effect was significant at intervention completion (mean Δ=.66, t(61)=2.686; p=0.01) and at 4-week follow-up (mean Δ=1.01, t(59)=4.546; p<0.001). There was no significant difference between the GR and IC group (mean Δ=0.54, t(41)=1.943; p=0.59). In addition, at follow-up, participants in the intervention groups combined agreed to a greater extent that 'sweet foods taste sweeter or too sweet after RESIST' (mean Δ=0.51 t(59)=1.996; p=0.05) in comparison to the control group. Overall, with a mean agreement score of 4.11 (SD=0.76), participants agreed the RESIST intervention booklet was easy to understand. All but one participant (98.4%) would recommend the intervention to a friend. A complete summary of outcomes is shown in **Appendix F14**.

Figure 7.7 Perceived benefits and acceptability of the RESIST protocol



A: Comparison of post-RESIST perceived satisfaction and benefits between the intervention groups (GR vs IC). Agreement reported on a 5-point Likert scale from 'Strongly disagree' to 'Strongly agree'; higher scores were indicative of higher agreement. All measures recorded at RESIST completion except for the 'Sweet food & drink taste sweet or too sweet' item which is reported at 4-week follow up.
B: Comparison of post-RESIST perceived satisfaction and benefits between the intervention group (GR and IC combined) and the WC. Agreement reported on a 5-point Likert scale from 'Strongly disagree' to 'Strongly agree'; higher scores were indicative of higher agreement. All measures recorded at RESIST completion except for the 'Sweet food & drink taste sweet or too sweet' item which is reported at 4-week follow up.
C: Comparison of post-RESIST barriers to adherence of the RESIST protocol between the active intervention groups (GR vs IC). Ease reported on a 5-point Likert scale from 'Very easy', 'Easy', Neither easy/difficult', 'Difficult', to 'Very difficult'; higher scores were indicative of greater difficulty.
* indicates $p \leq 0.05$.
Abbreviations: GR: Gradual and progressive sugar reduction group; IC: Immediate cessation sugar reduction group; WC: Wait-list control group.

7.5.7 Participant feedback

Participants were very enthusiastic about the study. Comments such as the following were typical: *"Sometimes I wasn't able to complete the task because I was at work and didn't have time to do so. That is the main reason why I wasn't able to complete the task every day. Apart from that I think it is a good way to monitor levels of sugar added to drinks.", or 'I'm so surprised at how quickly I adapted to no sugar. Been trying and failing to cut out sugar in my tea for most of my life, so thanks for helping me do that at last.".*

In the open comment sections of the questionnaires some study subjects mentioned they struggled to remember to complete the app task every evening *"I missed some sessions because I would forget to do it by the end of the day*". In addition, several participants mentioned they had experienced technical difficulties with the RESIST phone app in the early phases of the study*, "I never received notifications, so I filled in the tasks only when I remembered to in the first week of the intervention. Sorry!".*

Nevertheless, study subjects found the regular reminders beneficial: *"Great experiment! It was incredibly organised, and [the] regular updates and reminders were very useful.",* or *"This programme with the daily reminders has been great, although I originally found it hard to not add sugar to my hot drinks, I now cannot drink sweetened ones [anymore]. I have found that it has resulted in me eating sweet snacks less too, in the long run, which is good as I was trying to cut them down."*

7.5.8 Sample size calculation for a fully powered future trial

Power calculations were undertaken for the primary outcomes: (a) liking for sweetness in tea; and (b) daily sugar intake from tea. Results from sample size calculations are summarized in **Table 7.5** below.

Table 7.5 Required sample sizes for detection of intervention effectiveness on daily sugar intake from tea, and liking for sweetness in tea

Outcome	Comparison	Power	α	Observed η_p^2	Sample size[1]
Liking of sweetness in tea (T1,T3,T4)	time*condition	0.80	0.05	0.10	60
Daily sugar intake from tea (grams) (T1,T3,T4)	time*condition	0.80	0.05	0.08	75
Quit success (T3, T4)	time*condition	0.80	0.05	0.03	

[1] Power calculations were undertaken for two-factor repeated-measures ANOVA
Abbreviations: η_p^2= Partial eta squared

For a statistically significant effect of the intervention to be detected for differences in intervention effectiveness for the liking of sweetness in tea between the three conditions, a total sample size of 60 subjects is needed (~20 subjects per arm). Posthoc power analyses indicated that the current RESIST pilot trial was sufficiently powered (84%) to detect a difference between liking for sweetness in tea, supporting the conclusion that the RESIST protocol did not have an effect on liking for tea. The required sample size to detect significant change in daily sugar intake from tea was slightly greater. For this outcome, a total sample size of 75 was calculated (~25 subjects per arm).

7.6 Discussion

7.6.1 Summary of findings

The development and piloting of the RESIST protocol provides evidence that both a gradual and immediate cessation sugar-reduction protocol may reduce the intake of sugar in tea. Both intervention conditions were equally successful in terms of the proportion of participants who achieved total cessation of added sugar in tea (i.e. 'quitting') after completion of the 4-week intervention period compared to the control condition. Most importantly, reducing sugar in tea did not reduce overall liking of tea, suggesting that learned taste norms for sweetness can be selectively modified despite targeting only one type of added sugar. In addition, change in preference was achieved in a realistic time-period for a dietary intervention. Based on the preliminary analyses of the intervention effectiveness in this pilot study, a minimum sample size of 25 subjects per condition is required for a future fully-powered randomised controlled trial. Irrespective of whether gradual or immediate reduction protocols were followed, self-implemented reduction of added sugar in tea in combination with a smartphone-application was feasible, acceptable and effective in reducing the liking and intake of sugar in tea in a population of young adults. Satisfaction with the study materials and perceived effectiveness of the intervention was high, suggesting that longer-term behaviour change is possible.

1.1.2 Preliminary effectiveness of decreasing sugar intake in tea

Both the immediate and gradual sugar reduction groups appeared to decrease average daily sugar intake from tea from baseline to 4-week follow-up. Average sugar intake from tea reduced from 15.6 g/day to 8.4 g/day (-54%; -29 sugar kcal/day) in the GR group, and from 13.8 g/d to 6.5 g/day (-47%; -29 sugar kcal/day) in the IC group. WC maintained levels at 14.5g/day to 15g/day (+3%; +2 sugar kcal/day). These findings were significant at follow-up but not at 4-weeks, suggesting that participants in the intervention groups required longer than the 31-day intervention period to fully implement sugar reduction in their routine. Nevertheless, finding meaningful changes in sugar intake at 4-week follow-up suggests participants were motivated and able to sustain change.

The observed effect would translate to substantial dietary quality improvement. For instance, an individual who on average drinks two cups of tea per day with two teaspoons of sugar per cup would typically consume ~16g of sugar from tea daily (64 sugar kcal/day). In context, this equal half the amount of an adults daily total recommended free sugar intake (~30g/day) (World Health Organization 2015). Completion of the GR schedule would lead to an average 35 kcal reduction in energy intake from sugar per day, while completion of the IC schedule would result in an average reduction of 30 kcal/day (at 4-week follow-up). Over the course of a week, this reduction in calories consumed from sugar in tea would total approximately 245 kcal for the GR intervention and 210 kcals for the IC intervention. This effect is approximately equivalent to the number of calories in a normal-sized Mars bar (230 kcal; 51g) (Mars Inc 2016). This equates to 11,960 kcals over the course of a year which can add approximately 3.5 pounds of adipose tissue (3500 kcals leads to about one pound of fat) (Wishnofsky 1958).

The finding that the RESIST intervention reduced intake of sugar in tea was encouraging, and in line with previous research which investigated three different sugar reduction methods in coffee (Lenne & Mann 2017). In the coffee study, three conditions were investigated, and all three were successful in reducing calories consumed from sugar in coffee each day, after completion of a 14-day intervention period. Interestingly, the mindfulness-based intervention group and control group were most effective in this study, and the gradual reduction was least successful. Although, in this study, coffee was the target beverage and participants reported a greater average number of cups of coffee consumed per day (~4 coffees/day vs 2.7 teas/day), which explains the overall greater sugar reductions seen in this study compared to the RESIST intervention. In addition, sugar intake was reduced in smaller increments in the coffee intervention study, and the active intervention period was shorter at only 14 days (as opposed to 31 days in RESIST). Thus, the greater reduction in sugar intake may possibly be the result of smaller incremental reduction steps in the coffee GR condition, as it may be more effective and easier to implement. In addition, the control group in the coffee study was not a “true” control, given control group participants were instructed to reduce their sugar intake in coffee, and participants in this group were equally effective in reducing sugar intake as the mindfulness condition. Thus, it is not possible to establish whether the observed effects were attributable to the specific intervention condition protocol, or the result of participating in the research study overall.

The results from RESIST demonstrate that it is possible to reduce sugar in tea without impacting preference or intake of tea, suggesting preference modification for preferred level of sweetness may be feasible on a population scale for other food and drink products. Overall, 16 participants of the combined intervention conditions succeeded in quitting sugar in tea completely, and more encouragingly this effect was maintained at 4-week follow-up. Identifying effective interventions to reduce sugar intake in a lasting manner is important, as taste preferences are in-part shaped by dietary intake (Bertino et al. 1982; Bertino et al. 1986) and the concentration of tastant-level most commonly consumed (Methven et al. 2012). Taste preference modification therefore may be an upstream approach to reduce intake in the longer term. Stealth reduction of sodium in food supplies has been effective at the population level to reduce intake (Wyness et al. 2012) but empirical evidence for a similar strategy for sugar is not available. Evaluation of the program also confirmed that no compensatory addition of salt was added during home food preparation or at the table (Sutherland et al. 2013), and that the success of the UK salt reduction programme was uniform across the entire population (Millett et al. 2012). In a recent modelling study by Ma et al, it was estimated that a step-wise, gradual reduction in sugar of 40% in SSBs in the UK would lower dietary energy intake from sugar by 38.4 kcal/day after a 5-year implementation period (Ma et al. 2016). This provides some understanding of the potential that a gradual sugar reformulation programme may have as a wide-reaching strategy to reduce energy intake from free sugar in a targeted beverage type. However, the findings from RESIST did not provide any evidence of a shift in overall psychophysical preferred sweetness levels, but this was likely the result of being underpowered to detect small changes. Larger scale studies are needed to establish if the effects of a targeted sugar reduction campaign extend to also reduce general sweetness preferences for a variety of food and drinks.

1.1.3 Preliminary effectiveness of shifting sweetness preference for tea

This study found that within both the gradual reduction and immediate cessation sugar reduction intervention groups, the reduction of sugar in tea did not detrimentally affect preference for tea. Encouragingly liking for tea was unchanged in all groups at 4-week follow-up, supporting the observation that liking for unsweetened tea can be achieved after completion of the study period. Dietary preferences have previously been shown to be modifiable by alteration of overall dietary intake. Consumption of a low-sodium diet resulted in participants displaying greater sensitivity to saltiness perception of foods, and expressing greater acceptance of lower sodium foods (Bertino et al. 1982;

Bertino et al. 1986), while incidentally increasing sodium intake results in greater liking for higher sodium foods (Blais et al. 1986). Even though preference for sweetness is an innate human trait, large variation exists in preferred sweetness intensity and in the types of sweet food and drink items consumed (Reed et al. 2006). Liking for sugar is reinforced by frequent consumption of sweet tastes, in-part because repeated exposure to sweetness accustoms the palate to intensely sweet flavours (Bartolotto 2015). For instance, consumers of intensely sweet-tasting products show greater liking for sweeter beverages, and this greater affinity for intensely sweetened beverages is equally seen for frequent consumers of artificial sweeteners (Mahar & Duizer 2007). Alteration of sweetness preferences via change of total dietary intake remains uncertain. Modification of sweetness preferences was reported in a small-scale "Sugar and Artificial Sweetener Challenge" trial (Bartolotto 2015). In this study, volunteers (n=20) were challenged to eliminate all added sugars and artificial sweeteners from their diet for 14 days. Afterwards, most participants reported that sweet foods and drinks tasted sweeter (95%), and three-quarters (75%) of volunteers even reported that the effect of 'resetting' their palate to lower sweetness levels extended to other (non-sweet) foods. However, it remains uncertain whether restriction of intake of dietary sugars translates to shifts in changes in perceived pleasantness of sweetness. In a long-term RCT by Wise et al (2015), participants (n=16) were randomized to a low simple sugar diet for one month (40% replacement of dietary calories from simple carbohydrates with fats/protein) while participants in the control group were told to maintain their normal diet (n=17) (Wise et al. 2016). Sweetness perception during the intervention increased noticeably for added-sugar foods, yet change of total dietary sugar did not affect overall perceived pleasantness of sweet-tasting test foods and drinks (neither at intervention completion or over the course of 5-month follow-up).

In RESIST, intervention groups did not differ in overall preference for the lower sweetness tea after completion of the study. A comparable finding was demonstrated in a study with a similar design to the RESIST trial, a 16-week taste preference modification trial compared the relative effectiveness of gradual versus immediate cessation strategy in altering the preferred sodium levels in tomato juice. In this study by Bobowski et al (2015), participants were randomised to a group that either abruptly received the target low-sodium version of tomato juice from week 4 of the trial, while participants in the gradual reduction condition received juice reduced in sodium content in step-wise increments of 12%, reaching the same target low-sodium version of the tomato juice by week 14 (Bobowski et al. 2015a). However, during the sodium preference modification intervention, process measures of liking ratings substantially differed between the conditions. Within the immediate cessation group there was a significant decrease in liking just after the low sodium juice was consumed while liking was consistently maintained in the gradual reduction group. Participants in RESIST were all highly motivated to reduce sugar in tea, and were aware that the aim of the study was sugar reduction. In the study by Bobowski et al (2015), stratification of participants by motivation level revealed that highly motivated subjects were more tolerant of salt reduction, and that the intervention was more effective in this subgroup. This finding warrants follow-up in a future scaled-up version of RESIST, and agrees with previous research which has identified self-determined motivation as an important factor influencing the adoption of a healthful diet (Teixeira et al. 2011).

The one study that has demonstrated malleability of sweetness preferences in a hot beverage was the previously discussed sugar reduction intervention in coffee by Lenne et al (2017). Interestingly, this 14-day sugar reduction programme for coffee was also successful in gradually shifting sweetness preference levels for coffee while maintaining liking for the beverage. However, in contrast to the result seen in RESIST, maintenance of liking was not sustained in the GR group in the coffee trial. However coffee is very bitter and palatability of this beverage may depend to a much greater extent on the addition of sugar, compared to tea (Drewnowski 2009; Mennella et al. 2015). This may explain why liking was sustained in the GR condition in RESIST, but not in the GR coffee intervention. Additionally, duration and speed of progressive reduction of the respective active intervention periods differed. RESIST was twice as long as the trial by Lenne at al. (2017). Furthermore, progressive step-wise reduction steps were larger but more infrequent in RESIST, with sugar being reduced by 25% of the participant's baseline sugar amount every week. In contrast, in the sugar reduction trial for coffee progressive reduction occurred in increments of $1/6^{th}$ of the individuals' baseline sugar intake every two days. This difference in gradual reduction patterns would lead to a difference in dose of "exposure" to reduced sweetness versions of the beverage (i.e. two days exposure to each level of incremental sugar reduction in the coffee study compared to one week in RESIST) and may explain why enjoyment was not sustained for coffee but was maintained successfully for tea in both the GR and IC intervention groups during RESIST.

The few studies that have investigated sweetness preference change demonstrate that research on this topic is still in its early days. Alteration of taste preference can be accomplished by exposure to the flavour if taste intensity is changed from the most preferred level. However, the numbers of exposure encounters fluctuate depending on the food or drink item under consideration. Methven and colleagues suggested 3-5 consecutive exposures for acceptance of a reduced sodium version of soup to increase, while up to 14 exposures over the course of two weeks has been observed in children to achieve acceptance of a previously unknown fruit or vegetable (Fildes, van Jaarsveld, Wardle, et al. 2014; Cooke 2007). More research will be required to identify an optimal dose (as a function of intervention period*number of exposure occasions*magnitude of change) to effectively change sweetness preferences in the context of a single familiar dietary item. Moreover, more research is needed to identify whether a decrease in sweetness preference within one dietary item may be generalizable to other food or drinks, or diet overall. It may be that the innate preference for sweetness makes it especially difficult to change preferences for sweet foods and drinks.

1.1.4 Plausible mechanisms of sweetness preference modification

There are a few potential mechanisms to explain the findings of this study. Broadly speaking, modification of taste preferences can be explained by two distinct pathways: (1) the shift in perceived taste intensity, as measured by change in psychophysical level most preferred, and (2) the shift in perceived pleasantness, independent of a change in perceived taste intensity (de Graaf et al. 1996).

There was no significant change in preferred sucrose taste intensity (using the psychophysical test) following the RESIST intervention in either the GR or IC sugar reduction conditions. Nevertheless, the direction of change in psychophysical sucrose preference scores suggested that participants in the sugar reduction groups may have decreased their most preferred sucrose intensity level, and the small sample size of the participants undergoing the psychophysical taste test meant that the current study was underpowered to detect change in sweetness liking. A larger sample size will be needed to establish if GR and IC work to successfully reduce perceived sucrose pleasantness and whether there is any difference between the two intervention conditions. The potential decrease in sucrose liking may have been limited by conscious or subconscious compensatory increases in the consumption of sweet foods. This repeated exposure to other sweet foods or beverages may have prevented such alteration from occurring (Birch & Marlin 1982; Pliner 1982). Overall, the lack in change of preferred sucrose intensity means that the observed maintenance of tea enjoyment is likely explained by a shift in perceived pleasantness for unsweetened tea, independent of change in preferred level of sweetness. In addition, because participants were instructed to reduce the use of any sweeteners, both normal sugar and non-nutritive sweeteners, it is essential for future studies to identify if the exposure to sugar in tea or rather sweetness concentration per se would facilitate maintenance of enjoyment for unsweetened tea.

It is also possible that the chosen psychophysical measure of sucrose preference may not have been optimal to distinguish between individual variation in sweetness perception or sucrose preference (Bartoshuk 1991). Likewise, the range of sucrose concentrations that are used in the Monell two-series, forced-choice, paired comparison tracking technique are substantially higher that those observed in hot tea. For instance, a standard cup of tea (200 ml) with 1.5 teaspoon of sugar would be equivalent to 3g/100 ml (~3.0%) corresponding to the lowest concentration tested in the forced-choice procedure. It is known that individuals differ in their ability to detect sweetness, let alone change in sweetness levels, at low concentrations (Blakeslee & Salmon 1935) This mismatch in the range of sweetness level tested is a limitation that needs to be considered in the interpretation of the results of the RESIST intervention.

Taking the aims of **Study 4** into account, an alternative approach, the ‘just right/difference from ideal’ method, could have been considered. For this method, investigators require study participant to rate a sample as being sweeter or less sweet than their ideal of what the sample should taste like (Conner et al. 1988). A strength of this method is that every sweetness rating is relative to a personal ideal point, allowing precise assessments of an individual's ideal level of sucrose. This also allows estimation of individual tolerance of deviation from the ideal level of sucrose, making it a sensitive tool to measure change in preference over time, or as a consequence of a dietary modification intervention. In brief, the procedure for this method requires participants to rate 6-10 samples (either food or drink) of differing sweetness levels. Preferences ratings are made on a 100 mm linear scale, anchored by ‘At this sweetness level, I’d never eat/drink it’ or ‘At this sweetness level, I’d always eat/drink it’ on either extreme end. Samples are then presented to the participant, starting with a sample in the middle of the sweetness test range. In succession, participants are then asked to rate their liking of each samples level of sweetness, relative to the level of sweetness corresponding to their personal preference on the same linear scale. Subsequent sample are then spread evenly either side of the mid-point, thereby reducing the risk of range-frequency biases (Parducci 1963).

Nevertheless, while the sucrose levels in this method are closer to the range of a beverage such as tea, this method has mainly been used to investigate acceptability of reformulation of composite food the ‘just right/difference from ideal’ test measures sweetness liking and perception in more complex samples, by manipulating food and drink samples to contain slightly modified levels of sweetness. On the other hand, the Monell-forced choice taste procedure is designed to capture sweetness preference as an isolated taste, i.e. as sugar dissolved in water. Considering tea being the test drink in study 4, the forced-choice paired-comparison tracking procedure stood out as particularly appropriate. Importantly, rigorous research studies have provided evidence in support of this approach as a valid and reliable sweetness taste preference measure with strong real-world relevance (Mennella et al. 2011).

1.1.5 Feasibility and acceptability of the intervention

The Medical Research Council (MRC) specifies the need for intervention effectiveness, feasibility and acceptability to be identified during the pilot phase for a pilot-study to be scaled up to a fully-powered study (Craig et al. 2008). Feasibility is a key determinant of recruitment and retention rate estimation. As agreed on by Efficacy and Mechanism Evaluation (EME), Public Health Research (PHR), Health Technology Assessment (HTA) and Research for Patient Benefit (RfPB) Programmes, the aim of feasibility and pilot studies is to address the question: "*Can this study be done?*" (NIHR NETSCC 2017). In addition, the identification of the acceptability of an intervention program is recommended, given that when an intervention is considered acceptable, compliance, intervention fidelity and intended outcomes are more likely to be achieved.

Based on feedback from qualitative interviews and follow-up questionnaires, adhering to a pre-set sugar reduction schedule with daily reminders is feasible, easy to understand, and acceptable, but GR reduction appears to achieve higher satisfaction and is more engaging over the 4-week active reduction period. Nevertheless, participants from both groups benefited from partaking in the behavioural intervention. Insights from interviews (the focus of an MSc dissertation, as described in the Methods section) also suggested that gradual cessation may be a more acceptable way to increase cessation in the population, but it remains possible that abrupt quitting is the more effective method — even in persons who prefer not to.

No fundamental issues were identified with the acceptability and smoothness of the RESIST protocol. The high contact frequency (seen in in the GR condition) was in part reported as important to high retention rates. Additionally, the sense of 'community', although not explicitly emphasized as a part of the intervention, motivated participants to stick with their program. Detailed feedback from the qualitative RESIST participant interviews (n=15) was encouraging and informative, and various adaptions for a future study were proposed. Suggestions included: longer duration of the active intervention phase, in-app reminders of allocated sugar dose for the day, the ability to submit app responses multiple times a day, and adding the functionality for participants to declare when no tea was consumed on a specific day.

The intervention involved daily engagement with a smartphone application, which despite some technical difficulties, is vital to the delivery and acceptability of the study protocol. Smartphone technology for health is a growing field, with smartphone ownership growing continuously, and the use of apps is well-integrated into the lives of young adults (Buhi et al. 2013). Moreover, completion of the daily smartphone task also meant that participants self-monitored their tea drinking behaviour daily, requiring the individual to pay attention to their behaviour (Bandura 1998). Monitoring has been shown to be very effective for other health interventions (such as regular weighing in weight loss interventions) and might also explain the effect in the WC group. In summary, the RESIST intervention program was feasible, acceptable and effective, justifying the need for a fully-powered trial to establish if the GR or IC RESIST conditions differ in effectiveness. Moreover, observed effect sizes were substantial, adding further support that the RESIST programme has potential to achieve meaningful changes in dietary preferences and reductions in sugar intake from tea in the wider population.

1.1.6 Strengths and Limitations

First and foremost, the main strength of this study is the experimental randomised, controlled trial design, allowing the attribution of the observed reductions in sugar intake and the maintenance of preference for unsweetened tea to the intervention program. Additionally, the intervention was undertaken in free-living individuals in a non-laboratory setting, which relates closer to the real-world efficacy of the sugar reduction protocol. Also, participants were instructed to change only one dietary behaviour. The findings therefore provide practical knowledge on how sugar preference can be reduced to promote a more healthful diet in a real-word setting.

However, there are also limitations which need to be considered. First and foremost, the effectiveness of the intervention needs to be interpreted with caution. The RESIST trial was designed as an initial proof of concept study, and specifically aimed to assess acceptability and feasibility of the RESIST intervention protocol. Moreover, participants were all relatively young, lean and fairly healthy, so the results may not be applicable to older persons with a higher BMI, or those dealing with other health issues. Participants were also all highly motivated to reduce sugar in tea which is likely to have resulted in greater observed effects and acceptability of the intervention compared to less motivated populations.

Based on the design used, it was not possible to account for differences in time and exposure frequency to the ‘novel taste’ of less intensely sweetened tea in the GR group compared to the IC group. At intervention completion, participants in the IC group will have been repeatedly exposed to the unsweetened taste of tea for 31-days, while participants in the GR group will have been exposed to the completely unsweetened taste of tea on only eight occasions. Additionally, study participants were not stratified by motivation level, or by level of hedonic sensitivity which in a previous salt-reduction trial have been shown to be associated with changeability of salt liking (Bobowski et al. 2015a). Future studies should consider incorporating such study features to further investigate important gaps in the field of sweetness preference modification.

Finally, apart from the psychophysical sweetness preference test, all measures relied on self-report. These data may be subject to recall bias and social desirability bias. Even though blocked randomisation was undertaken at baseline for intervention group allocation, residual confounding can never be entirely ruled out. Additionally, because of the nature of the intervention, it was not possible to blind participants or the researchers to the sugar reduction condition individuals had been allocated to. It is possible that pre-conceived views may have biased outcomes (Sibbald & Roland 1998). Nevertheless, this is the first taste modification study to include both sweetness preference, intake and psychophysical measures of taste preference change, and provides an unparalleled wealth of data to study the mechanism of sweetness preference change.

1.2 Conclusions

The results of this pilot study suggest individuals may successfully reduce the amount of sugar consumed in tea using two different behavioural strategies – gradual or immediate cessation of sugar in tea. Promisingly, reducing sugar in tea didn’t appear to affect liking. Demonstrating that repeated exposure can lower preferred levels of sugar in foods is of importance given that on a population-level consumption of free sugar greatly exceeds levels recommended for general health and weight maintenance.

This is a timely study, with high relevance for public health. There is growing concern about the level of sugar intake in the UK population, especially in drinks; highlighted by the UK soft drink industry levy, which is due to take effect in April 2018. The study showed that it is possible to successfully reduce a common form of sugar intake – sugar added to tea. The intervention conditions were simple, short, and low in intensity with potential for roll out on a larger scale via public health initiatives.

These data provide a promising foundation to scale-up and refine a sugar-reduction protocol fit to support the needs of the general population. Policies for sugar reformulation are typically met by resistance from the food and drink industry due to concerns about reduced consumer acceptance. Typically non-nutritive sweeteners are used as a strategy to meet consumer demands of palatability but sweeteners are still limited in their application due to structural limitations, sub-optimal aftertastes, and consumer concerns about potential detrimental health effects (Suez et al. 2015; Wang et al. 2016). Providing evidence that it is possible to 'retrain' consumers' palates to prefer food and drink formulations with lower levels of sweetness may help convince the food and drink industry to take affirmative action.

In conclusion, individuals can successfully reduce the amount of sugar consumed in tea using two different behavioural strategies – GR or IC. Reducing sugar in tea doesn't affect liking, suggesting long-term behaviour change is possible. A fully powered randomised controlled trial is needed to confirm findings, assess longer-term outcomes, and establish if GR and IC differ in effectiveness.

Chapter 8.

Concluding discussion

8.1 Introduction

Overconsumption of energy-dense foods and drinks are implicated as risk factors for overweight and a range of negative health outcomes. One of the most important determinants of consumption of a particular food or drink is *liking* for that food or drink (Brunstrom & Shakeshaft 2009; Hebden et al. 2015; Phan & Chambers 2016; Story et al. 2002; Lennernäs et al. 1997; Domel et al. 1993; Domel et al. 1996; Drewnowski et al. 1999; Biloukha & Utermohlen 2001; Kourouniotis et al. 2016; Glanz et al. 1998). This thesis aimed to develop a reliable questionnaire measure of preferences for a comprehensive range of food and drinks for older adolescents, and to investigate how liking for specific food groups or drinks is cross-sectionally associated with adiposity. Additionally, analyses were undertaken to identify the origin of individual differences in food and drink preferences, providing insights into their potential modifiability during this developmental phase. Previous food and taste preference modification interventions have been successful in increasing acceptance of unfamiliar or disliked fruits and vegetables in children, and decreasing liking for high sodium food in adults, but evidence for the alteration of other taste preferences is limited, and there is little research in young adults. Based on finding strong influence from the wider-environment on food and drink preferences, a sweetness preferences modification intervention was developed to reduce liking for and intake of sugar in tea in young adults.

To achieve these aims, food and drink preference data were collected from a population-representative twin birth cohort, the Twins Early Development Study (TEDS), when the twins were 18-19 years old. The genetically-sensitive nature of a twin study, and the narrow age-range of the participants enabled me to establish the relative importance of genetic and environmental influences on food and drink preferences during older adolescence.

The following chapter provides a brief overview of the key findings of the thesis and discusses the theoretical, practical, and methodological strengths and limitations of the presented work. A summary and discussion of the important implications of the research concludes the chapter.

8.2 Summary of thesis findings

The topics in this thesis were explored using four main research aims to guide the research, as laid out in detail in **Chapter 2**. The aims of this thesis were addressed across **Studies 1-4** (**Chapter 4-7**). The results relating to each objective are summarised below.

8.2.1 Aim 1

To develop a food and drink preferences questionnaire that comprises a wide range of food and drink items relevant to common dietary choices of older adolescents, and to broadly characterise patterns of food and drink preferences

The development of a comprehensive food and drink preferences questionnaire was described in **Study 1** (**Chapter 4**). The questionnaire items were updated from a 96-item parent-report questionnaire of children's food preferences. The question format was adapted to allow older adolescents to self-report their dietary preferences via an online version. Food items were modernised to be representative of the foods and drinks most commonly consumed in the current food environment (e.g. addition of hummus, avocado), and were tailored for adolescent dietary habits (e.g. removal of jelly and ice lollies, addition of peanut butter and tinned tuna). Drink items were added as these had been largely omitted from previous dietary preference questionnaires. A questionnaire including 69 food items and nine drink items was pilot tested (n=124), and test-retest results showed that liking for these items was reasonably stable over a period of two weeks. Principal components analysis revealed six traditional food categories underlying food preferences (vegetables, fruits, meat/fish, dairy, snacks and starches), replicating the structure seen previously in young children. Groupings reflected logical food categories, and suggested young adult's preferences do not simply cluster by taste profile (e.g. sweet or salty foods). The findings from **Study 1** culminated in the development of a reliable and cost-effective psychometric tool to measure food and drink likes and dislikes in young adults. After exclusion of a few food items that did not fit well into one of the six food groups, the final questionnaire included 63 food items and nine drink items.

Snack foods were the most preferred and vegetables the least liked food groups. On the other hand, orange juice was the most popular drink and non-nutritive sweetened beverages were rated as the least liked. This pattern of preferences for food groups was expected, with similarly higher liking observed for energy-dense and highly palatable foods in older adolescents, as has previously been observed in children. In line with this finding, the most highly rated drink, orange juice, is sweet and energy dense. However, orange juice also provides a good source of nutrients, and encouragingly the nutrient-rich (but still sweet) fruit category was the second most liked food group. Taken together, the observed patterns of older adolescents' food and drink preferences showed a distinct affinity for sweet foods and drinks, while nutrient-dense and lower energy vegetables were least liked. These dietary preference patterns are not consistent with dietary guidelines recommended for optimal general health.

8.2.2 Aim 2

To establish the relative contribution of genetic, shared-, and non-shared environmental influences on food and drink preferences in older adolescence

In **Study 2**, the relative importance of genetic and environmental influences on food and drink preferences was explored using the data collected with the questionnaire developed in **Study 1.** Individual differences in food and drink preferences were found to have a moderate genetic basis in older adolescents, with the remaining variation driven by non-shared environmental factors.

Heritability estimates were of a similar magnitude to those observed in previous adult and paediatric twin studies of food preferences but this was the first such study in older adolescents and the first in any age-group to explore preferences for a range of non-alcoholic drinks. In twin studies of young children, a consistent and sizeable influence of the shared environment is demonstrated which is not seen in adults. The findings from **Study 2** identified no shared-environmental influence on liking for food categories or for drinks, likely reflecting the gains in independence experienced at this life-stage and greater influence of social, societal and commercial pressures.

8.2.3 Aim 3

To examine the relationship between a comprehensive range of food and drink preferences and adiposity in a large sample of older adolescents.

Study 3a explored the cross-sectional associations between food and drink preferences and BMI. Previous studies investigating the association between dietary preferences and adiposity have primarily focused on a narrow range of foods, mostly concentrating on liking for foods high in fat and/or sugar, or exclusively on liking for fruits and vegetables. This study investigated the relationships between the full range of questionnaire-measured food and drink preferences and BMI in older adolescents. Higher liking for typically energy-dense food groups or beverage types was not positively associated with higher BMI. Similarly, higher liking for lower energy-dense foods (e.g. vegetables) and drinks (e.g. non-nutritive sweetened beverages) were not inversely associated with BMI. In fact, higher liking for NNSBs was significantly positively associated with BMI, although the effect size was small. A small but significant positive association was also observed between greater liking for dairy foods BMI. The findings emphasise that any potential relationships between dietary preferences and adiposity are likely to be complex.

A more sophisticated study design was also used to investigate the cross-sectional relationship between food and drink preferences and adiposity in **Study 3b**. All MZ twins who were very discordant for BMI (<3 BMI units) were identified and their food and drink preferences directly compared. Because MZ twins share 100% of their genetic material and 100% of their shared environment, studying the association between food preferences and adiposity allows researchers to completely control for any confounding by shared environmental factors (e.g. SES and ethnicity) and genetic influence (e.g. pleitropy). The heavier twin compared to the leaner twin showed greater liking for fruit. However, when all MZ twins were included in the analysis (not only the pairs who were very discordant), this within-pair difference was not associated with greater BMI-discordance. These findings indicate that food and drink preferences are not robustly related to adiposity in older adolescents. Food preferences may not be the most important driver of actual intake in this age group, *how much* rather than *what* we eat may be more important for BMI in older adolescence.

8.2.4 Aim 4

To establish the feasibility, effectiveness and acceptability of a behavioural taste preference modification intervention to reduce preference for, and intake of, sugar in tea.

Study 4 described the development and findings of a pilot study comparing the feasibility, acceptability and effectiveness of a sweetness preference intervention. The study was called the REduction of Sugar In tea STudy (RESIST), and consisted of a self-implemented behavioural modification schedule instructing participants to either gradually reduce or immediately eliminate the addition of sugar in hot tea relative to a control group. This study built on the evidence from the previous studies in the thesis which showed non-shared environmental influences were the most important shaper of drink preferences in older adolescents, suggesting potential modifiability of these preferences. The results from the 4-week randomised controlled trial suggested that participants effectively reduced sugar intake from tea without any loss of enjoyment.

Both the gradual and immediate cessation sugar reduction protocol were well-received and appreciated by study participants, suggesting that learned taste norms for sweetness can be selectively reduced in a familiar drink, without targeting other aspects of dietary sugar intake.

The intervention materials consisted primarily of simple booklets which could be disseminated via e-mail and a free smartphone application. The cost for the delivery of the RESIST materials was therefore low. Feedback from semi-structured qualitative interviews provided insightful yet low-cost suggestions about how to increase the functionality and acceptability of the intervention program. A future fully-powered trial of at least 75 participants (~25 participants per trial arm) will be required to confirm these preliminary findings and to establish if the gradual or immediate cessation conditions differ in effectiveness for the reduction of sweetness liking in tea, and intake of sugar from tea.

8.3 Implications for theory and future research

The findings of this thesis have advanced our understanding of the aetiology of food and drink preferences and their relationship with adiposity, yet substantial gaps remain. Important new directions for future research have been identified and are discussed in detail below.

8.3.1 Measurement of food and drink preferences

Traditionally food and drink preferences have been investigated using questionnaires focused on a limited range of items, presumed to be dietary risk factors for poor health (e.g. fatty and sweet foods). Consequently, the aetiology and impact of preferences for a broader range of food and drink items have never been studied.

The reliable and low-cost psychometric questionnaire developed in **Study 1** provides a much-needed[l] comprehensive tool to measure food and drink preferences in large populations of adults. In addition, it provides a solid theoretical structure to further study the associations between preferences for key food groups and drinks in relation to adiposity, or other health outcomes. This will aid the identification of important food

[l] At time of thesis submission (April 2018), 24 separate researchers had reached out to obtain permission to use the food and drink preference questionnaire from **Study 1** for their own research. A copy of the publicly available version of the questionnaire and accompanying scoring sheet is shown in **Appendix G1.**

likes or dislikes that stand out as risk factors for poor dietary health, which can be selectively targeted in future interventions (Story et al. 2008).

The literature review in **Chapter 1** identified that the measurement and investigation of drink preferences has been particularly neglected. This is an oversight, as drinks are progressively becoming important contributors to total dietary energy intake (Nielsen & Popkin 2004; Drewnowski et al. 2013; Stern et al. 2014; Ford & Dietz 2013). Further research is required to develop a more comprehensive version of the drink preference questionnaire presented in **Study 1**. In particular, the inclusion of energy drinks, smoothies, and a wider variety of other hot beverage options needs to be considered. Not only are some of these beverages very energy-dense, but older adolescents are the biggest consumers of these intensely sweetened drink types (Heckman et al. 2010; British Soft Drink Association 2016). It would also be beneficial to consider a similar factorial reduction technique as used for the grouping of food preferences to empirically derive the underlying dimensions of drink preferences (Newby & Tucker 2004). The factorial structure of drink preferences has yet to be investigated and it is important to understand if drink preferences cluster by taste profile, mouth feel, or like food preferences, group into logical 'traditional' categories (Redondo et al. 2014).

8.3.2 Linking food and drink preferences and adiposity

Few cross-sectional associations between food and drink preferences with adiposity were found in this thesis. Only higher liking for dairy food and for NNSBs was positively associated with higher BMI across the whole sample, although not in within-family analyses – suggesting these associations may be confounded by shared environmental factors (such as SES and ethnicity) or genetic influences on both. While these results suggest that dietary preferences are not key correlates of adiposity at this age, it also highlights the complexity between preferences for certain foods and drinks and BMI.

Longitudinal studies in larger population samples that study how liking for dairy foods and NNSBs in childhood predict adult body weight are necessary to further understand the directions of the relationships. The observational evidence linking dairy fat intake and adiposity remains inconsistent (a full discussion on the literature linking fat preference and adiposity is described in section **Study 3**.). However, **Chapter 1** identified 5/6 studies in adults, and 3/8 in children which reported significant associations between greater liking for fatty and sweet food and drink types and greater adiposity. Closer inspection of the items in the 'dairy' category of the questionnaire developed for this thesis reveals that items are high in fat (butter, cream, mayonnaise) or taste sweet (custard, yoghurt). Additionally, all items shared a generally smooth consistency, suggesting that mouth feel might be implicated in determining the liking for the items loading on to the 'dairy' factor. This is important, as it provides further insights into the possibility that the human can detect free fatty acids as a distinct taste sense, possibly a key aspect influencing individual differences in liking for dairy foods. This 'fat' sense, termed 'oleogustus', may soon be considered a sixth distinct primary taste modality (Running et al. 2015). Additionally, it will be essential to determine the direction and strength of the association between the preference for fatty foods and variation in adiposity, to rule out reverse causality of adiposity status and dairy food preferences.

The observed positive association between higher liking for NNSBs and BMI, and a null association between liking for SSBs and BMI, demonstrates the complex nature of drinking behaviours and energy balance. Intake of the latter has been causally linked to obesity, while NNSBs are energy neutral and therefore cannot directly contribute to caloric intake. However, frequent consumption of artificially sweetened NNSBs is a common dieting strategy to curb cravings for sweet foods to manage or reduce body weight (Dennis et al. 2009; DellaValle et al. 2005). Moreover, there remains considerable uncertainty with regards to the biological or psychological effects of artificial sweetener consumption that may relate the liking and frequent consumption of NNSBs to higher energy intake or overweight (Lohner et al. 2017). This emphasizes the need for continued research to understand the social, commercial, physiological and psychological factors that influence the liking for sweetness, especially in beverages (Bobowski et al. 2016; Mennella et al. 2016).

8.3.3 Identifying genetic influences on food and drink preferences

There was a small to moderate genetic influence on individual differences in preferences for both non-alcoholic drinks and food in older adolescents. Overall, genetic influences were slightly stronger for food than for drink preferences, suggestive of greater potential for environmental intervention to modify drink preferences. This thesis additionally confirmed that genetic influences on food preferences previously observed in studies of children likely persist into adulthood. However, genetic and environmental influences on food or drink preferences have never been studied in the same sample across these developmental phases (i.e. using longitudinal data in the same twin sample). Collecting further food preference data from the Gemini and TEDS twin study as the children reach adolescence and adulthood would help shed light on whether the heritability of food preferences do indeed track across the life course.

While moderate heritability estimates for both food and drink preferences were seen across all dietary preferences, the magnitude of genetic influences differed across food and drink categories. Among food groups, genetic factors were strongest for preferences for fruit and vegetables, and among drinks, for fruit squash, NNSBs and tea. It is important to emphasise at this point that stronger heritability of a psychological trait or behaviour does not implicitly render behavioural intervention futile; it merely suggests that it may require additional efforts to change certain dietary preferences compared to others. For example, simply increasing the availability of a particular food within a person's environment may be insufficient to increase liking if preference for that food is under strong genetic influence. This is especially important when considering the higher heritability estimates for variation in preference for the healthy and nutrient-dense food group vegetables. However, communicating this knowledge to policy makers and those in charge of distributing meals to older adolescents (e.g. in college) may be helpful. If these stakeholders understand that they are working to overcome a genetic tendency of youngsters to be less accepting of vegetables, this might encourage individuals to persist with additional strategies to improve acceptance, such as incentivising consumption.

Given substantial genetic influences were consistently seen for older adolescents' food and drink preferences, it would be valuable and interesting to advance our understanding of the mechanisms involved. Early research to elucidate the biological pathways shaping variability in dietary preferences relied on gene-association studies. A handful of genetic variants that explained variation in bitterness perception (Bufe et al. 2005), sugar consumption (Eny et al. 2010), and sucrose taste sensitivity were identified (Fushan et al. 2009). However, these are all aspects of taste sensitivity and contribute to variation in dietary preferences via genetic variation in taste receptor units. Only one recent GWAS in 4611 individuals has identified important genetic variants related to the behavioural expression of *food* preferences (distinctly more complex traits) (Pirastu, Kooyman, Robino, et al. 2016). Importantly, none of the genes identified were known to code for taste receptors, indicating genes impact food preferences in ways other than simple differences in taste perception. Given the mounting evidence and technological advances, the logical next step to advance the field is a large-scale GWAS of food <u>and</u> drink category preferences. This approach is the first step to identify genetic polymorphisms that function via biological pathways other than variability in taste receptor units, providing valuable insights into the biology determining dietary preferences beyond taste. Moreover, this would also provide important insights into whether the genetic variants that influence food preferences overlap with drink preferences, or whether these dietary preferences have distinct genetic origins. This could also be explored using a multivariate twin model.

1.2.1 Identifying environmental influences on food and drink preferences

Study 2 indicated that the established shared environmental influence (aspects of the environment shared by twin pairs such as the early home food environment) on food preferences in childhood has been displaced by factors in the wider environment by older adolescence. This has important implications for policymakers concerned with improving the nutritional health of the population. In the current permissive food environment where food and drink options are diverse and continuously available, dietary preferences are likely pivotal in determining intake (Eertmans et al. 2001). The sizeable non-shared environmental influence on food and drink preferences in older adolescents highlights the potential for environmental modification of preferences for unhealthy foods and beverages through national public health programs. Such public health policies targeting the wider environment are promising techniques to bring about substantial population level health gains (Briggs et al. 2017). Public health policy should prioritize action to implement legislation to tackle established detrimental influences on food choice, including stricter and wider-reaching regulation of food marketing targeted at older adolescents, regulation of price promotions of junk foods, and fiscal measures to discourage the purchase of SSBs and other high sugar and/or high fat processed foods (McGale et al. 2016; Hawkes et al. 2015).

The results from **Study 3a and b** suggest the contribution of an individual's food and drink preferences to their adiposity status is limited. Moreover, the results from **Study 3b** demonstrated that no food or drink preferences were associated with adiposity in genetically-matched individuals. Clearly further investigation to identify the salient factors that explain adiposity discordance in genetically identical individuals is needed. Limited evidence has tentatively implicated differences in eating behaviours, dieting attempts and body dissatisfaction as important contributors to BMI variability. However, finding that the '*what*' of older adolescents' dietary preferences is not important for body adiposity discordance raises the possibility that '*how much*' and '*why*' people eat are more important drivers of body weight. Both portion size and meal frequency are aspects of eating behaviour shown to contribute to excess weight gain in early childhood (Syrad et al. 2016). More research is therefore needed to investigate if published dietary guidelines may be more effective in the prevention of excess weight gain if information is expanded to focus on age-appropriate meal structure and portion size.

The RESIST intervention (**Study 4**) may help shed light on the consistent finding of a substantial non-shared environmental influence on all categories of food and drink preferences in **Study 2**. A caveat of twin studies is that it is not possible to disentangle whether non-shared environmental influence is attributable to non-shared factors in the macro-environment (e.g. food advertising), or factors in the more proximal environments (e.g. friends, partners or work colleagues). Interestingly, a key factor contributing to compliance, reported by the RESIST participants in the qualitative interviews was the importance of feeling ‘part of a social group’. This knowledge positively reinforced engagement with the sugar reduction intervention via creating a sense of social support and social comparison despite the intervention being delivered to the individual. This supports the importance of social norms for older adolescents’ dietary behaviours and preferences, highlighting manipulation of the perception of social norms as a plausible mechanism for shifting dietary preferences towards more healthful choices in this age group (Templeton et al. 2016; Sharps & Robinson 2017).

Taken together, findings from the studies in this thesis emphasise the value of interventions targeted at both the individual and the wider-environment. More importantly, these approaches are not mutually exclusive, i.e. there is scope for individual-focused interventions to improve dietary preferences within wider-environmental legislation, combining forces to shift dietary preferences towards more healthful choices.

8.3.4 Intervention work on the modification of food and drink preferences

This thesis provides novel evidence in support of the malleability of sweetness preferences in a familiar beverage, without the loss of enjoyment, and without restriction of other sweet foods or drinks. The intervention protocols developed in **Study 4** provide an acceptable, cheap and feasible approach to reduce the intake of sugar from tea without affecting overall enjoyment of tea. Participants were instructed to either gradually or immediately eliminate the addition of sugar to tea over a period of 31 days. Throughout the intervention, participants were told to track their daily tea and sugar intake via an app. Participants overall found the instructions easy to understand, but satisfaction was greater in the GR group. However, the GR condition schedule involved greater contact frequency from the researcher, as this condition received regular reminders when the next sugar reduction step was due. More personalised contact may improve intervention success and compliance, but this could result in a greater rule complexity and participation burden in the intervention (Fry & Neff 2009). A practical alternative may be the provision of an online forum or network so that intervention participants can support each other (Eysenbach 2004), This approach has previously been shown to be favourable in weight loss or physical activity interventions in young adults (Napolitano et al. 2013; Turner-McGrievy & Tate 2013; Valle et al. 2013).

The findings from **Study 4** also provide the first quantitative insights on the sizeable contribution of sugar from tea to dietary intake in some British young adults. On average, participants in the RESIST intervention consumed 14.6 g/day (SD=8.7) of sugar in tea, close to the entire daily recommended amount of free sugar intake for a healthy adult (World Health Organization 2015). The substantial contribution from added sugar in tea to total sugar and energy intake emphasises this add-on as a targetable and modifiable dietary behaviour. In recognition that calories from hot beverages are a targetable risk factor for poor diet, the CDC has adapted their weight loss guidelines to emphasize the importance of cutting calories from drinks, specifically addressing the unseen amount of sugar in hot beverages purchased in coffee shops (The Center for Disease Control and Prevention 2015). Informing coffee/tea consumers of the nutritional and health implications of beverage add-ins could help them make informed and healthier diet choices.

Importantly, the RESIST proof of concept study demonstrated that ceasing to add sugar to tea is possible while maintaining the enjoyment of tea. However, not all participants were successful in achieving quit status. Other factors that will be important to assess in relation to intervention success are genetic and phenotypic measures of sweetness sensitivity. For instance, genetic variability in *TAS1R2* affects habitual consumption of sugars and has been implicated in variation in response to dietary interventions (Eny et al. 2010). Results from a weight-loss RCT found participants classified as ‘sweet likers’ or ‘sweet dislikers’ (measured using a behavioural tasting task) differed in the magnitude of reduction of caloric beverages when water was the recommended substitution. Future studies should explore how a person’s sensory tasting profile may mediate the effectiveness of a sugar reduction protocol such as the one used in **Study 4** (Turner-McGrievy et al. 2016). Related to this, participants in the psychophysical sweetness preferences sub-study did not report a shift in their hedonic appreciation of sucrose. More work is needed to investigate if sweetness preference modification interventions work via shifting the sweetness preferences perception threshold level, or by shifting the threshold level that is rated as the most preferred (De Graaf & Zandstra 1999).

The alteration of sweetness preferences in tea may also affect sweetness preferences for other food and drink items. Given the established correlation between preferences for various sweet tasting foods, and the shared genetic pathway underlying a common sweetness preference factor, it is likely that the reduction in liking for sweetness in tea may shift preference thresholds for sweet food and drinks more generally (Keskitalo, Tuorila, et al. 2007; Hwang et al. 2015). Intervention follow-up was undertaken at four weeks post-intervention in RESIST. An important future research question also concerns the persistence of sweetness preference change at longer-term follow-up, and if reduced sugar in tea is maintained long term without the intervention.

Methods for planned health behaviour change can be roughly classed into two main approaches: (i) a planned abrupt change or cessation, i.e. the ‘cold turkey approach’, or (ii) a gradual, progressive change process with a pre-established endpoint as the goal. For smoking, the abrupt cessation approach has been experimentally established as the most effective quitting strategy and dominates as the main recommendation; for changes in dietary behaviours there is an inclination to recommend the gradual elimination of alcohol, salt or sugar (Lindson-Hawley et al. 2016; Bobowski 2015). Two previous experimental studies of smoking cessation have directly compared the effectiveness and acceptability of these two different approaches, with abrupt cessation being more effective (Lindson-Hawley et al. 2016). On the other hand, gradual reduction of sodium was more efficient for liking of dietary sodium (Bobowski et al. 2015a). The findings from **Study 4** provide insights on the effectiveness of the RESIST intervention to not affect liking for tea using both approaches, but a scaled-up trial to test for significant differences in effectiveness for change in sugar intake from daily tea will be necessary.

8.4 Strengths and limitations

Taken together, this thesis builds on previous research exploring the origins and plasticity of food and drink preferences, as well as their impact on adiposity. Food and drink preferences were measured using a reliable, stable and comprehensive psychometric measure. A great strength of this psychometric tool was the inclusion of multiple drink preference items, providing novel insights on the aetiology of this important but understudied aspect of energy intake.

A further advantage of the studies in this thesis was the focus on older adolescents and young adults. Data for **Studies 1, 2** and **3** were collected from TEDS a large UK population representative cohort of 18-19-year-old twins. The intervention in **Study 4** was developed and tested in a population of university students of similar age. Overall, a variety of methods were used to address the various aims of the thesis set out in **Chapter 1**. A combination of quantitative and qualitative analyses, as well as observational and experimental data provides a detailed characterisation of dietary preferences implicated in dietary intake and health.

The limitations relevant to the individual studies in this thesis have been discussed in detail in their respective chapters. In the following section, limitations that apply more generally to the thesis are considered.

8.4.1 Measurement issues

The empirically-derived six-factor structure of food preferences obtained in **Study 1** provides a broad measure of the likes and dislikes of British older adolescents. However, this structure might not capture dimensions of food preferences in other populations. Preferences were rated on 5-point Likert scales, which may have lacked sensitivity to detect sufficient variation, especially for the most popular items such as chocolate or orange juice. Consequently, ceiling effects may have resulted in the loss of statistical power. This is especially important in the context of a twin study as this method relies on the decomposition of variation in a measured trait to quantify its aetiology. However, none of the food category scores had particularly low standard deviations, suggesting sufficient variance to allow accurate SEM modelling. An improvement on the Likert scale approach could be the use of a *general Labelled Magnitude Scale* (gLMS), as it has been shown that these measurement scale are better at detecting minor fluctuation in preference scores and are more suitable for across group-group comparisons of liking for different food groups (Kalva et al. 2014).

One weakness of the food and drink questionnaire related to the limited range of beverage types included (seven non-alcoholic and two alcoholic beverage types). While this represented one of the most comprehensive measures of drink item preferences to date, the range was limited in comparison to the variety of food items. It was therefore not possible to identify robust drink preference factors comparable to those for food preferences, which would have been more reflective of underlying drink preference dimensions and less sensitive to outliers. Specifically relating to the measurement for the liking of fruit squash, an important oversight was the failure to distinguish between sugar-sweetened or artificially sweetened fruit squash. Given the substantial evidence for genetic influence on sweetness perception, distinguishing between drinks with added sugar or added high potency sweeteners would help to clarify how the complexity in human perception of sweetness shapes drink preferences (Keskitalo, Knaapila, et al. 2007; Keskitalo, Tuorila, et al. 2007; Hwang et al. 2015). Despite all the strengths of the food and drink preferences questionnaire, it also did not consider any short-term restrictions in an individual's diet, which may have influenced cravings and preferences on the day of data reporting (Griffioen-Roose, Mars, et al. 2012; Griffioen-Roose, Hogenkamp, et al. 2012).

There were some weaknesses in the design of RESIST (**Study 4**) which may have biased interpretations. First and foremost, participants were not blinded to the purpose of the study. It is possible that performance bias (i.e. knowledge of the intervention aim) may have inflated effectiveness of study outcomes. As data on compensatory sugar intake lacked detail, it is not possible to rule out changes in dietary intake or other behaviours which may have knowingly or unknowingly occurred in response to reduction in sugar intake in tea. Nevertheless, change in number of cups of tea per day as well as change in add-ins (non-nutritive sweeteners and milk/creamer) were measured and these didn't differ throughout the intervention.

8.4.2 Self-reported data

A limitation that warrants consideration was the reliance on self-report for both food and drink preferences and height and weight measures used to calculate BMI. Social desirability bias may have occurred if individuals over reported liking for healthy foods and drinks, and under-reported liking for unhealthy food or body weight in an attempt to gain societal approval (Grimm & Grimm 2010). Social desirability bias is a particular issue in questionnaire studies relating to health behaviours and weight (Hebert et al. 1995). Under-reporting of dietary intake has been shown to increase with BMI and such systematic underreporting distorts observed associations (Bratteby et al. 1998). This may therefore have contributed to the lack of association between food and drink preferences and adiposity observed in this thesis. However, dietary preference data were collected via an anonymised online platform which may have reduced the magnitude of this effect.

Another issue relating to the use of self-reported data could be misreporting of height (over-estimating) and weight (under-estimating) measurements. Due to the high economic cost, organisational cost, and participant burden of objective height and weight measurement, self-reported anthropometrics provide a pragmatic alternative. Ideally, accuracy of self-reported BMI could be verified by comparison with objective measures yet the economic and logistic issues precluded this option. However, previous studies have provided support for the validity of self-reported height and weight in young adults (Kuczmarski et al. 2001; Spencer E.A. et al. 2002; Goodman et al. 2000; Brener et al. 2003).

8.4.3 Cross-sectional data

The measures used in **Study 1, 2,** and **3** were all cross-sectional. Heritability estimates of food and drink preferences and their associations with adiposity need to be interpreted keeping this limitation in mind. Investigating the longitudinal aetiology of food and drink preferences in the same sample would be required to confirm if the absence of a shared environmental influence during older adolescence is maintained into adulthood. Because heritability is a time-specific index, and the aetiology of behaviour is known to vary with age, future studies using longitudinal data from the same sample are needed.

Moreover, the relationships between food and drink preferences and adiposity in **Study 3** cannot be adequately understood using cross-sectional data. Most notably the observed positive association between greater liking of NNSBs and BMI hints at potential reverse causation or dieting behaviours misrepresenting cross-sectional associations. Similarly, for the positive association between liking of dairy food and BMI, it cannot be concluded whether greater liking for foods elicited higher adiposity status, or if higher adiposity increased the liking for the taste of dairy foods, and subsequent intake. The nature of bi-directional relationships between taste preferences and body adiposity remain uncertain (see **Chapter 6** for a detailed discussion) (Donaldson et al. 2009). Longitudinal studies in population representative samples will be essential to gain better insights into whether dietary preferences are a cause or consequence of adiposity.

8.4.4 Representativeness of the samples

The TEDS cohort and RESIST sample were predominantly lean, highly educated and healthy young people. However, previous studies have shown that the TEDS sample is broadly representative of key socio-demographic characteristics in the UK (Haworth et al. 2008). Rates of overweight and obesity were lower in the TEDS sample compared to rates for this age group in the general UK population (NHS Digital 2015). Nevertheless, the studies in this thesis were undertaken in samples broadly representative of the target population, meaning the findings are likely to be indicative of the UK's older adolescent population. However, it is possible that there are distinct food and drink preferences that characterise more extreme weight. Future research on food and drink preferences in older adults, including those of a much higher weight, would clarify whether there are aberrations in food and drink preferences at the higher end of the adiposity spectrum.

A further limitation regarding the recruitment methodology used was the reliance on financial incentives to encourage high questionnaire completion rates in **Study 1**, and to encourage enrolment, engagement, and low attrition in the intervention in **Study 4**. This may have resulted in selection bias, as the content of the study invitations mentioned the topic of the research (food preferences) and thereby may have selectively attracted participants favourably disposed to health or dietary research participation. Caution is warranted before the results of the study are generalised to larger, more diverse, and less health-conscious populations.

Lastly, generalizability of the findings from twin studies to the wider population has been questioned. However, concerns regarding the representativeness of twins for a phenotype such as food and drink preferences are low. Population-representative data on food and drink preferences in older adolescents are not available and so it is not possible to formally test representativeness. However, there is empirical evidence that twins are generally born earlier, and have lower birth weights than singletons (Evans & Martin 2000). However, other research has shown that these weight differences between twins and singletons have largely disappear by the time twins turn 3 years of age (van Dommelen et al. 2008).

8.4.5 Twin study design assumptions

The interpretation of findings from twin studies is based on a number of critical assumptions, and violation of these compromise the validity of the results. Central to the twin method is the ‘Equal Environment Assumption’ (EEA), which assumes that MZ and DZ twins are equally exposed to the same environmental influences relevant to the trait under study (Kendler et al. 1993). MZs may in fact share some aspects of their environments to a greater extent than DZs, including aspects of the environment that could influence food and drink preferences. If this is the case, the heritability estimates may be inflated. In particular, critics of the twin method have claimed that MZ twins are treated more alike socially, more frequently share friendship groups, share a room, and are treated more like a unit; this could result in their exposure to more similar food occasions (Evans & Martin 2000; Joseph 2000). Similarly, it is possible that MZ twins may have experienced more similar in-utero flavour environments (if they shared a placenta, as do two thirds of MZ twins), given that DZ twin pairs always have separate placentas. These factors may have resulted in environmentally-inflated resemblance between MZ twin pairs being ascribed to genetic factors, resulting in inflated heritability estimates (Hettema et al. 1995). However, it is unlikely that differential antenatal flavour exposure has a strong effect on food and drink preferences in older adolescence (Cooke & Fildes 2011). Additionally, a recent systematic review of twin studies found that outcomes relating to birth weight are most likely affected by chorionicity (whether twins share the foetal membrane layer within the amniotic sac; indicator of a shared placenta) and that any effect of pre-natal exposure is of greater magnitude early on, and lessens during development (Marceau et al. 2016). Assessment of the validity of the EEA is complex, however a comprehensive pooled analysis of 11 twin studies found no issue relating to this assumption for the validity of a range of characteristics, supporting twin methodology (Bouchard Jr. 1997).

Lastly, potential overestimation of observed heritability because of possible gene-environment correlation needs to be considered. Genotype-environment correlation is the phenomenon whereby an individual with a genetic predisposition towards a particular environmental exposure actively seeks out that type of environmental exposure, which further strengthens the genetically influenced trait. For example, individuals with a greater liking for a specific food or drink type are likely to express behaviours that increase the likelihood of them receiving or having access to these foods or drinks. This phenomenon increases resemblance for that trait between more genetically-alike individuals – i.e. MZ twins relative to DZ twins (Plomin et al. 2013) – which increases heritability because the difference between MZ and DZ pairs increases. This may have affected the results from this thesis and should be considered in future longitudinal follow-up analyses.

8.5 Conclusion

Food and drink preferences are one of many factors contributing to the intricate network of factors influencing dietary choices, and ultimately adiposity. Confirming previously established heritability of categories of food preferences in paediatric twin studies, the findings from this thesis extend these findings by showing substantial genetic contribution to variation in both food and drink preferences in older adolescents. Crucially, the strong shared environmental influences observed for children's foods preferences are no longer important by the time adolescents begin to make autonomous dietary preferences, indicating that interventions to encourage healthful diets are best directed at the wider environment.

In a world of unprecedented obesogenic pressures within the food environment, developing the necessary protective legislative measures is a public health priority, yet effective implementation will require time. Meanwhile, food and drink preference modification programs provide a complementary approach for targeting motivated high-risk individuals. Low fruit and vegetable intake, energy dense snack food and sugar-sweetened beverage consumption are important, preventable mass exposures.

Unhealthy dietary preference patterns contribute to the selection of foods suboptimal for nutritional health. However, the relationship between food and drink preferences and body adiposity in older adolescence remains unclear. The finding that sugar intake and sweetness preferences can be altered by targeting exposure in a hot beverage, is promising and encouraging for food industry, policy-makers and the individual consumer. Overall, the findings of this thesis suggest that targeting both the wider environment as well as the individual can act together to influence cultural, political and individual-level shifts towards improved food and drink preferences of young people as they embark on adulthood. This has the potential to reduce negative health consequences later in life.

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Appendix A.

Chapter 1 - Literature review on food and drink preferences

Appendix A1 Studies investigating the association between food and drink preferences, and measures of adiposity

	Food and drink preference measurement methods	Foods or Drink items	Food and drink preference measurement methodology details	n	Weight/Adiposity measures	Age	Country	Design	Results
Food neophobia and liking for fruits and vegetables are not related to Italian children's overweight (Laureati et al. 2015)	Laboratory taste test	• Fruit: apple, pear, grapes and miyagawa-citrus fruit. • Vegetables: fennel, radish, broccoli and carrot.	~40 g of fruit and vegetable was served raw to the children immediately prior to their mid-morning snack. Liking scores were measured using a 7-point hedonic facial scale	n=528 n=257 (M) n=261 (F)	Researcher measured BMI z-scores Under weight: n=8 (1.5%) Normal weight: n=369 (69.9%) Overweight: n-=132 (25.0%) Obese: n=19 (3.6%)	6-9 y	Italy	Cross-sectional	Pearson's correlation coefficients for BMI and the liking of: Pear: r=0.05 Apple: r=0.03 Miyagawa citrus: r=-0.01 Grapes: r=0.00 Broccoli: r= 0.00 Carrot: r= 0.05 Fennel: r= -0.02 Radish: r=-0.01 No significant relationship between BMI and food liking
Preferred sweetness of a lime drink and preference for sweet over non-sweet foods, related to sex and reported age and body weight (Conner & Booth 1988)	Forced choice test questionnaire Laboratory sensory test Questionnaire	Forced choice between: • Celery/carrot • Tomato juice/orange juice • Cheeseboard/cake trolley • Plain milk/ flavoured milk • Soda water/tonic or lemonade • Bread & margarine: plain/honey or chocolate spread	Sweet vs. non-sweet forced choice questionnaire Objective palatability rating of sugary drink sweetness intensity Frequency of sugaring tea and coffee	n=344	Not reported	6-65 y	UK	Cross-sectional	BMI did not correlate significantly with any of the sweetness measures (r= -0.30 to 0.21, p>0.05).
From motivation to behaviour: A model of reward sensitivity, overeating, and food preferences in the risk profile for obesity (Davis et al. 2007)	Questionnaire	72 common food items • High-fat preference: Mean preference scores for 36 high-fat items • High-sugar preference: mean preference score for 24 high-sugar items	The Food Preference Questionnaire (Geiselman et al. 1998) Preferences scores were measured using a 9-point hedonic scale	n=151 (F)	Researcher measured BMI (% of sample) Underweight BMI: n=1 Health BMI: (38.5%) Overweight BMI: n=41 (27%) Obese BMI: n=51 (34%) 27% were overweight (BMI ≥25 and <30); and 34% were obese (BMI≥30). One subject had a BMI of 18.4.	33.5 y (mean) Range: 25 – 50 y	Canada	Cross-sectional	Preference scores for fatty foods was significantly correlated with BMI (r=0.18, p<0.05) Preference scores for high sugar foods were positively associated with BMI(r=0.13) but failed to reach statistical significance.

	Food and drink preference measurement methods	Foods or Drink items	Food and drink preference measurement methodology details	n	Weight/Adiposity measures	Age	Country	Design	Results
Adiposity is not associated with children's reported liking for selected foods (Hill et al. 2009)	Questionnaire	• Fruits: apples, bananas, oranges, tangerines, grapes, melon, peaches, pears, plums, apricots, strawberries, and raspberries • Vegetables: broccoli, cabbage, carrots, cauliflower, green beans, leeks, courgettes, mushrooms, onions, parsnips, peas, sweetcorn, salad, peppers, tomatoes, sprouts, turnips, cucumber, celery, spinach, swedes, yams, plantain, vegetables snacks, and baked beans. • Fatty/sugary foods: plain biscuits, chocolate biscuits, cakes, fruit pie, ice cream, ice lollies, yoghurt, crisps, chocolate, sweets, chips, and pizza	Food preferences questionnaire 5 point Likert scale	n=366	BMI SD scores, waist-SD scores and fat mass index Underweight BMI SD score (n=56): −1.78 (0.49) Lower healthy weight BMI SD score (n= 120): −0.69 (0.37) Higher healthy BMI SD score (n=123): 0.69 (0.42) Overweight BMI SD score (n=49): 1.81 (0.28) Obese BMI SD score (n=18): 2.88 (0.23)	7-9 y	UK	Cross-sectional	Mean food category liking was similar across the weight groups BMI SD score did not significantly predict liking for fatty or sugary foods (F(1, 364) = 0.27, p=0.60, R^2 = 0.001), vegetables (F(1, 364) = 2.39, p=0.12, R^2 = 0.007) or fruit (F(1, 364) = 0.05, p=0.82, R^2<0 .001))
Fat preferences, dietary fat intake and body composition in children (Ricketts 1997)	Laboratory forced choice sensory test	• Regular or low fat brownies • Regular or low fat peanut butter cookies • Regular or low fat chocolate cake • Regular or low fat chocolate chip cookies	Hedonic rating of high and low fat snack foods. 9 point Likert scale	n=88 n= 51 (M) n= 37 (F)	BMI, triceps skinfold test, and subscapular skinfold test.	9-12 y	USA	Cross-sectional	Fat preference scores correlated significantly with BMI (r=0.51, p<0.056) and triceps skinfold body fat percentage (r=0.46, p<0.05) but did not reach significance (r=0.34) in relation to subscapular body fat.

	Food and drink preference measurement methods	Foods or Drink items	Food and drink preference measurement methodology details	n	Weight/Adiposity measures	Age	Country	Design	Results
Fat preferences and fat consumption of 3- to 5-year-old children are related to parental adiposity. (Fisher & Birch 1995)	Laboratory sensory test	• High-fat food items: Cheese, peanut butter, chicken nuggets, and margarine • Low fat food items: Ham, wheat bread, peas, carrot sticks, banana, wheat roll, tuna fish, broccoli, unsweetened applesauce and corn	Taste and taste ranking procedure Preferences were indicated by the participant pointing to either a smiley, neutral or frowning face	n=18	Mean weight-for stature percentile: 50th (SD=7)	3-5 y	USA	Cross-sectional	Children's fat preference and triceps skinfold measurements correlate significantly ($r = 0.61$; $p < 0.01$) Correlation coefficients for children's fat preference and weight for stature ($r=0.35$) and subscapular skinfold % ($r=0.30$) were not significant but in the expected direction
Taste preferences and body weight change in Japanese adults: the JPHC Study. (Matsushita et al. 2009)	Questionnaire	• Do you like rich and heavy food? • Do you like sweet food?	4 point Likert scale ('dislike', 'neither dislike nor like', 'like')	N=29 103	Self-reported weight Self-reported recalled weight gain >5kg from age 20 to baseline questionnaire (yes/no)	40-59 y at baseline		Prospective cohort (10 y follow-up)	Odds ratio (OR) of gaining $\geq$ 5 kg weight was significantly higher for 'likers' of rich and heavy taste compared to 'dislikers': Men: 1.45 (95% CI: 1.31, 1.61) Women: 1.28 (95%CI: 1.16, 1.41) OR of gaining $\geq$ 5 kg weight was significantly higher for female 'likers' of sweet taste compared to 'dislikers', but not for males: Men: 1.00 (0.91, 1.11) Women: 1.22 (1.09, 1.36) Non-significant association of changes in body weight and the liking of rich and heavy foods: Men: β=0.15 (s.e. = 0.07) Women: β=0.14 (s.e. = 0.07) Significant association ($p \leq 0.01$) of changes in body weight and the liking of sweet foods: Men: β=0.33 (s.e. = 0.07) Women: β=0.24 (s.e. = 0.05)

	Food and drink preference measurement methods	Foods or Drink items	Food and drink preference measurement methodology details	n	Weight/Adiposity measures	Age	Country	Design	Results
Taste preferences in association with dietary habits and weight status in European children: results from the IDEFICS study (Lanfer et al. 2012)	Laboratory forced choice sensory test	Forced-choice testing • Fat preference: Low fat (8% fat) vs. high fat (18% fat) cracker • Sweetness preference: Low sugar apple juice (0.53% sucrose) vs high-sugar (3.11% sucrose) apple juice	Taste preference rank test	n=1696	Researcher measured height and weight Overweight BMI z-score: 23.35%	6-9 y	Italy, Estonia, Cyprus, Belgium, Sweden, Germany, Hungary and Spain	Cross-sectional	High versus low preference for high fat crackers was significantly associated with increased odds for overweight or obesity (OR=1.8, 95%CI: 1.3-2.5). High versus low preference for sweetened apple juice was significantly associated with increased odds for overweight or obesity (OR=1.15, 95%CI: 1.1-2.1).
Surveying Food and Beverage Liking (Duffy et al. 2009)	Questionnaire	23 food and drink items: • Fibre-rich foods: asparagus, blueberries, broccoli, cantaloupe, carrots, grapefruit juice, oatmeal, strawberries, sweet potato, tomato juice, and whole wheat bread. • Fatty foods: Beef steak, butter, cookies, French fries, fried chicken, icing, mayo, and sausage.	Food preference questionnaire Hedonic Labelled Magnitude Scale (gLMS), anchored by 'strongest imaginable like' and 'strongest imaginable dislike'.	n=88 (F)	Self-reported height and weight Healthy BMI: n=38 Overweight BMI: n=24 Obese BMI: n=14	Female: 25-55 y	USA	Cross-sectional	Greater preference for fat was significantly associated with BMI (r=0.25, p<0.05). Greater preference for fibre-rich food was also significantly associated with BMI (r=0.26, p=0.04).
Food preferences of 10- to 14-year-old boys and girls (Diehl 1999)	Questionnaire	• 114 food items e.g. pizza, ice cream, spaghetti, french fries, hamburgers, pudding, corn flakes, potato chips, popcorn, common fruits, liver, canned or steamed fish, raw sauerkraut and red cabbage. • 14 beverages	Food preference questionnaire 5-point facial hedonic scale	n=1233 n=696 (M) n=537 (F)	BMI z-score	10-14 y	Germany	Cross-sectional	For boys: Preference scores for 6/114 items were negatively associated with BMI z-scores, i.e. sweet pancakes, cake, cookies, chocolate, chocolate bars and boiled sweets (all p≤0.01) For girls: Preference scores for 4/114 items were positively associated with BMI z-scores, i.e. semolina pudding, muesli, grapefruit and hazelnuts (all p≤0.01).

	Food and drink preference measurement methods	Foods or Drink items	Food and drink preference measurement methodology details	n	Weight/Adiposity measures	Age	Country	Design	Results
Correlation between a liking for fat-rich foods and body fatness in adult Japanese: a gender difference. (Nakamura et al. 2001)	Questionnaire	Preference score for: • Fat-rich foods • Butter or lard • Fat-rich meats such as beef steaks or hamburgers	Preference ratings on a 4 point Likert scale ('yes, very much' to 'no')	n=892 n=473 (M) n=419 (F)	Researcher measured height and weight, waist circumference and skinfold thickness Male: 22.8 kg/m^2 Female: 20.9 kg/m^2	Male: 29.5 (5.3) Female: 26.4 (3.9)	Japan	Cross-sectional	Males reporting their preference for fat-rich foods as "yes, quite a bit" or "yes, very much" had higher BMI scores, waist to hip ratios and higher values of SFT[1] (all $p<0.001$). Association between the higher liking of fat-rich food preferences and change in BMI or change in waist were not significant. Females reporting their preference for fat-rich foods as "yes, quite a bit" or "yes, very much" had higher BMI scores ($p<0.001$) and higher values of SFT[1] (all $p<0.0001$). Associations between the higher liking of fat-rich food with WHR[1] ratios, SFT[1], change in BMI or change in waist were not significant. After adjustment for lifestyle factors, associations between the liking of fat-rich foods were significant for BMI ($\beta=1.42$, $p\leq0.001$), WHR[1] ratio ($\beta=0.019$, $p<0.001$) SFT[1] ($\beta=0.071$, $p\leq0.001$) and ASFT[1] ($\beta=0.22$, $p\leq0.001$) in males. After adjustment for lifestyle factors, associations between liking of fat-rich foods were significant for BMI ($p=0.003$) & SFT[1] ($p=0.006$) in females. Liking for fat-rich foods explained 7-9% of the variation in BMI, WHR[1], SFT[1], and ASFT[1] in males. Liking for fat-rich foods explained 2-6% of the variation in BMI and SFT in females Liking for butter/lard was significantly associated with BMI ($p<0.01$), WHR[1] ($p<0.01$) and ASFT[1] ($p<0.01$) in males. Liking for fat-rich meat was significantly associated with WHR[1] ($p<0.01$), ASFT[1] ($p<0.01$) and SFT[1] ($p<0.01$) in males. Only preference for fat-rich meats and SFT[1] was significant ($p=0.04$) in females.

	Food and drink preference measurement methods	Foods or Drink items	Food and drink preference measurement methodology details	n	Weight/Adiposity measures	Age	Country	Design	Results
Food preference questionnaire as a screening tool for assessing dietary risk of cardiovascular disease within health risk appraisals. (Duffy et al. 2007)	Questionnaire	19 food items including • Fatty foods: mayonnaise, cheddar cheese, beef steak, gravy, butter, milk chocolate, sausage, Sweets, fried chicken, sour cream, and whole milk. • Sweet foods: milk chocolate, sugar whipped cream, and sweets • Bitter beverages and foods: coffee, grapefruit, cooked broccoli	Food preference questionnaire Hedonic Labelled Magnitude Scale (anchored by 'strongest imaginable like' and 'strongest imaginable dislike')	n=422 (M)	Researcher measured height, weight and waist circumference	Mean: 46 y	USA	Cross-sectional	Increased preference for fat was significantly positive associated with BMI (r=0.11, p<0.05) waist circumference (r=0.15, p<0.05).
Tracking of toddler fruit and vegetable preferences to intake and adiposity later in childhood (Fletcher et al. 2017)	Questionnaire	Liking of fruit and vegetable items. • 11 vegetables: Carrots, tomatoes, baked beans, peas, cabbage, lettuce, cucumber, onions, okra, yam and gourd. • 7 fruits: oranges, apples, banana, tinned peaches, mangoes, lychees and guava.	Parent-completed food preference questionnaire based on the Survey of the Diets of British School children (Department of Health 1989) Ratings on a 5-point Likert scale ('dislikes a lot' to 'likes a lot') Number of vegetables, fruits and FV combined liked was calculated	At 30 months: n=456 At 7-8 years: n=550 Longitudinal sample: n=346	Researcher-measured BMI, bioelectrical impendence fat and skinfold Z-scores	Age at food preference questionnaire: 30 months Age at adiposity measurements: 7-8y	UK	Longitudinal	Number of fruits and vegetables liked at 30 month was not related to BMI, bioelectrical impendence fat and skinfold Z scores at age 7–8.

	Food and drink preference measurement methods	Foods or Drink items	Food and drink preference measurement methodology details	n	Weight/Adiposity measures	Age	Country	Design	Results
Black Children with High Preferences for Fruits and Vegetables Are at Less Risk of Being at Risk of Overweight or Overweight (Lakkakula et al. 2008)	Questionnaire	Liking of fruit and vegetable items • 17 fruits: grapes, apple, orange, strawberry, watermelon, pineapple, pineapple, peaches, pears, banana, plums, kiwi, tangerine, cantaloupe, mango, apricots, papaya, avocado • 21 vegetables: french fries, corn, lettuce, sweet potato, baked potatoes, potato salad, carrots, green beans, greens, cabbage, broccoli, peas, tomatoes, cucumber, celery, spinach, bell pepper, garlic, onion, coleslaw, cauliflower.	Items based on the 'Fruit, Juice, and Vegetable Availability Questionnaire' (Marsh et al. 2003) Ratings on a 3-point Likert scale ('I don't like this' to 'I like this a lot') Mean score for fruit and vegetable preferences was calculated	341	Researcher-measured BMI	9-11y.	USA	Cross-sectional	Children who had very low preferences for fruits and vegetables were 5.5x more likely to be at risk of overweight or overweight compared to children who reported high preferences for these foods ($p<0.01$). Preference scores for fruits ($p=0.13$) only or vegetables only were not associated with weight status ($p=0.70$).

[1] *Abbreviations: SFT=Subscapular skin thickness, ASFT=Abdominal skinfold thickness, WHR=Waist-to-hip ratio.*

Appendix A2 Family studies investigating resemblance of parent-child food and drink preferences

	Food and drink preference measurement method	Foods or Drink items	Food and drink preference measurement methodology	n	Age	Country	Design	Results
Similarities in food preferences between children and their siblings and parents (Pliner & Pelchat 1986)	Questionnaire	139 food items: • "Single" foods (e.g. various fruits, vegetables, meats), • "Mixed" foods (e.g. lasagna) • Condiments (e.g. ketchup).	Food preference questionnaires were completed by the mother of all families Preference ratings were collected using a 6-point Likert scale For adults: "likes and eats", "dislikes and eats", "likes but does not eat", "dislikes and does not eat" and "other". For children: "likes and eats", "dislikes and eats", "likes but does not eat", "dislikes and does not eat", "never offered" and "offered but never tasted".	n=55 families	Children: 24-83 months	Canada	Cross-sectional	Children's food preferences were more similar to those of members of their own (real) families than to those of 'pseudo families' ($p<0.001$). Children were more alike to their siblings, than their mothers or fathers ($p < 0.001$).
Food preferences in families (Logue et al. 1988)	Questionnaire	55 foods and food groups • Milk and milk products • Green and yellow vegetables • Citrus fruits • Potatoes, vegetables, and other fruits • Meat, poultry, fish, and eggs • Bread, flour, and cereal • Butter and margarine • Single items: Chili pepper, coffee, tea, and alcoholic beverages	9-point Likert scale ('dislike extremely' to 'like extremely') Additional question regarding: What ethnic type(s) of food were you raised on? (e.g. American, Italian, Greek, etc.) Put more than one if applicable." The ratings for all of the foods within each factor were summed to create ten factor scores.	n=77 students Siblings, mothers and fathers & students n=241	Probands: 15.7 y	USA	Cross-sectional	Correlations were calculated for each of the ten factor scores and for each of the six types of relative pairs (mother-father, mother-proband, father-proband, mother-sibling. father-sibling, proband-sibling), for a total of 60 correlations. 19/60 correlations were significant ($r=0.24$-0.55; $p<0.001$). The greatest number of significant correlations was the mother-proband comparison, for which 5 out of 10 correlations were significant ($p<0.001$), whilst only 1/10 for father-proband comparisons were significant. The greatest number of significant correlations (4/6) was for resemblance in the liking of 'spices' ($p<0.001$), whilst none of the correlations for the factor 'fruits' were significant.

	Food and drink preference measurement method	Foods or Drink items	Food and drink preference measurement methodology	n	Age	Country	Design	Results
Family resemblance in food preferences (Pliner 1983)	Questionnaire	47 food items (representing four unspecified food groups)	7-point Likert scale	n=105	Probands: 19 – 24 y	Canada	Cross-sectional	Small positive correlations (r=0.25 for mother-child pairings; r=0.25 for father-child pairings) for food item preference scores that had been reduced to four broad food groups
Family resemblance in attitudes to foods (Rozin et al. 1984)	Questionnaire	22 items that were selected to included strong-tasting and strange foods: • Radishes, beef, liver, yogurt, diet soda, black olives, black coffee, soft-boiled eggs, sweet pickles, peppermint candy, lima beans, liverwurst, banana, chicken curry, sandwich with hot peppers, raw clams, tongue, Limburger cheese, cow pancreas, cow heart, cooked lizard, raw daisy, and cooked eel.	9-pont Likert scale (extremely dislike' to 'extremely like')	n=34	Probands 17 - 23 y	USA	Cross-sectional	Parent-child resemblances on food preferences were quite small (range: -0.035 to 0.460, mean: 0.152) Preference scores for black olives showed the highest and only significant correlation (r = .501, p<0.05).
Parental influence on the child's food preference (Burt & Hertzler 1978)	Interview for children Questionnaire for parents	32 items; grouped into four broad groups	4-point Likert scale	n=46 children	Children: 5-6 years	USA	Cross-sectional	Mother and fathers food preferences influence the child's food preferences equally
The relationship between children's food preferences and those of their parents (Birch 1980)	Interview	8 items (including fruits, vegetables, sandwiches, and snacks)	3-point Likert scale	n=128 children	Children: 2 y 11 months – 5 y 7months	USA	Cross-sectional	10% of the mother-child resemblance and 6% of the father-child correlations were significant (p < 0.05).
Family Consumption of Low Fat Foods: Stated Preference Versus Actual Consumption (Weidner et al. 1985)	Phone interview	5 low fat, low cholesterol foods: • Rice, yogurt, refried beans, broccoli, and fish	5-point Likert scale	n=30 children	Children: 8 – 11 y	USA	Cross-sectional	Resemblance of mothers and children's food preferences for each of the assessed foods were insignificant (r= -0.09 to .28, p>0.05).

	Food and drink preference measurement method	Foods or Drink items	Food and drink preference measurement methodology	n	Age	Country	Design	Results
Children's food preferences: a longitudinal analysis. (Skinner et al. 2002)	Interview	Mothers: 196 items + 3 alcoholic beverages Children: 90 items Items were grouped in to the following categories: • Vegetables (cooked) • Vegetables (raw) • Fruits, • Juices, • Meats/poultry/fish, • Meat mixtures, • Meat alternatives (e.g. legumes) • Eggs • Dairy • Breads • Cereals • Desserts • Soft drinks	3-point Likert scale	n=70 children	Children: 8 y	USA	Longitudinal	Correlations were strongest for foods rated as 'liked' by the children (r=0.37, p=0.0014), foods disliked by the children, (r=0.25, p=0.03) and for foods that previously had never been tasted (r=0.24, p=0.04).

Appendix A3 Twin studies investigating the relative genetic and environmental contributions to food preferences

	Foods items	Methods	MZ/DZ	Age	Nationality	Design	Findings	Comments
Heritability studies of single food item preferences								
Family environment, not heredity, accounts for family resemblances in food preferences and attitudes: A twin study (Rozin & Millman 1987)	• 15 food items: Radishes, beef liver, black olives, black coffee (unsweetened), soft boiled eggs, lima beans, liverwurst, raw onion, peppermint lifesaver, plain sugar, plain yogurt, lemon juice or lemon (unsweetened) and spicy foods • How hot do you like food spiced with chilli pepper?	Telephone interview Ratings on a 9-point hedonic scale 'How much do you like…?'	MZ pairs: n=19 DZ pairs: n=18	17-26 y	Philadelphia, USDA	Cross-sectional	Differences between rMZ and rDZ only significant for peppermint, yoghurt and "how hot do you like food spiced with chilli peppers?" Indication of a genetic component to the liking of chili pepper. Negative correlations for the liking of peppermint and yoghurt prohibits a sensible interpretation of these results.	Limited sample size unlikely to detect small genetic effects
A twin study examining the genetic influence on food selection (Krondl et al. 1983)	• 24 individual food items: Unsweetened grapefruit juice, green beans, broccoli, apple juice, unsweetened orange juice, strawberries, bacon, ham, turnips, doughnuts, honey, ice cream, jam, potato chips, brussels sprouts, cabbage, cauliflower, spinach, lemon juice, unsweetened apple sauce, beer, black coffee, black tea and tonic water	Taste sensitivity, preference and use of 24 foods	MZ pairs: n=13 DZ pairs: n=10	39.2 (12.0) Range: 19-58 y	Toronto, Canada	Cross-sectional	Significant difference in rMZ and rDZ preference scores for the liking of 8/24 food items were detected (incl. green beans)	Limited sample size unlikely to detect small genetic effects
A twin study of personal preferences. (Faust 1974)	• Is there any food you really dislike? Do you like [Yes/No]: • Melon • Tomatoes • Parsnips • Cheese • Yoghurt • Anchovies • Liver		MZ pairs: n=48 DZ pairs: n=48	17-40 y	UK	Cross-sectional	Only significant ($p<0.05$) difference between MZ and DZ spontaneous food dislikes, was for spicy/foreign food. Only significant ($p<0.05$) difference in listed food likes by MZs and DZs was seen for melon.	

	Foods items	Methods	MZ/DZ	Age	Nationality	Design	Findings	Comments
Evidence for a genetic influence on preference for some foods (Falciglia & Norton 1994)	• 17 food items were tasted and rated: cereal (sweetened), snack cake, soda/cola, cereal (unsweetened), french fries, spaghetti, beans, broccoli, corn, apple, orange juice, American cheese, chicken, cottage cheese, hamburgers, low fat milk and whole milk.		MZ pairs: n=14 DZ pairs: n=21	9-18 y	Cincinnati, Ohio	Cross-sectional	Significant differences in MZ and DZ preference score ICCs were found for 6/17 food items: orange juice ($p \leq 0.05$), broccoli ($p<0.05$), cottage cheese ($p<0.05$), chicken ($p<0.05$), sweetened cereal ($p<0.05$), and Hamburgers ($p<0.10$).	All twins were still living at home
Twin study of heritability of eating bread in Danish and Finnish men and women (Hasselbalch et al. 2010)	• Total bread intake • White bread use • Rye bread use • Preference for rye bread (proportion of rye bread consumption relative to total brad consumption)	Frequency of eating half a slice of rye bread, a slice of wheat whole grain bread or a slice of white (wheat) bread: • Never • 1 time/month • 2–3 times/month • 1–2 times/week • 3–4 times/ week • 5–6 times/ week • 1 time/day • 2–3 times/day • 4–5 times/ day • 6–7 times/day • ≥8 /day	MZ: n=233 pairs DZ: n=342 pairs (Danish GEMINAKAR) MZ: n=663 DZ: n=1346 (Finnish cohort)	18-67 y (GEMINAKAR) 22-27 y (Finnish cohort)	Denmark/Finland	Cross-sectional	**GEMINAKAR (M)** A: 0.37 (0.27 – 0.46) E: 0.63 (0.54 – 0.73) **GEMINAKAR (F)** A: 0.27 (0.18 – 0.34) E: 0.74 (0.66 – 0.82) **Finnish cohort (M)** A: 0.48 (0.33 – 0.60) E: 0.52 (0.40 – 0.67) **Finnish cohort (F)** A: 0.29 (0.14 – 0.43) E: 0.71 (0.57 – 0.86)	Consumption of bread as well as choice of bread is influenced by genetic predisposition. Environmental factors shared by the co-twins have no significant effects on bread consumption and preference in adulthood.
Heritability studies of categorical food preferences								
Comparison of Socioenvironmental Factors in Monozygotic and Dizygotic Twins, Testing an Assumption (Smith & Vandenberg 1965)	• Staple foods (chicken, ham, pork chops, steak (beef), liver, fried eggs, boiled eggs, mashed potatoes, raw potatoes and seafood) • Fruit (apples, oranges, peaches, pears, grapes, plums, bananas, apricots, watermelon) • Vegetables (raw carrots, tomatoes, celery, radishes and cucumber)	Self-reported 4 point Likert scale	MZ pairs: n=61-63 DZ pairs: n=51-54	13-16 y	Baltimore, USA	Cross-sectional	Differences between rMZ and rDZ only significant for mean vegetable and fruit category preference scores	Limited sample size unlikely to detect small genetic effects

	Foods items	Methods	MZ/DZ	Age	Nationality	Design	Findings	Comments
Nature and nurture in children's food preferences. (Fildes, van Jaarsveld, Llewellyn, et al. 2014)	114 food items (75 items retained in the PCA) • Vegetables • Fruit • Protein (meat and fish) • Dairy (cheese, yogurt, and eggs) • Starches (rice, bread, and pasta) • Snacks (chocolate, cookies, ice cream, and chips)	Parent-reported liking of their child's liking for specific foods 5-point Likert scale	MZ pairs: n=458 DZ pairs: n=872	3 y	UK	Cross-sectional	**Vegetables** A: 0.54 (0.47 – 0.63) C: 0.35 (0.27 – 0.42) E: 0.11 (0.10 – 0.13) **Fruits** A: 0.53 (0.45 -0.61) C: 0.35 (0.26 – 0.43) E: 0.13 (0.11 – 0.15) **Protein** A: 0.48 (0.40 – 0.57) C: 0.37 (0.27 – 0.45) E: 0.15 (0.13 – 0.17) **Dairy** A: 0.27 (0.20 – 0.35) C: 0.54 (0.47 – 0.60) E: 0.19 (0.16 – 0.22) **Snacks** A: 0.29 (0.24 – 0.35) C: 0.60 (0.54 – 0.65) E: 0.11 (0.09 – 0.12) **Starches** A: 0.32 (0.26 – 0.38) C: 0.57 (0.51 – 0.62) E: 0.11 (0.10 – 0.13)	
Heritability of food preferences in young children. (Breen et al. 2006)	• Vegetables (broccoli, cabbage, carrots, cauliflower, green beans, mushrooms, onions, parsnips, salad greens and tomato) • Desserts (cream, cakes, pastries, fruit pie, sponge pudding, custard, dairy desserts) • Meat and Fish (beef, lamb, pork, chicken, bacon, fried fish, white fish, oily fish) • Fruit (apples, bananas, citrus fruits, grapes, peaches, strawberries and fruit juice)	Parent-reported liking of their child's liking for specific foods 5-point Likert scale	MZ pairs: n=111 DZ pairs: n=113	4-5 y	UK	Cross-sectional	**Vegetables** A: 0.37 (0.20 – 0.58) C: 0.51 (0.27 – 0.42) E: 0.13 (0.10 – 0.13) **Fruits** A: 0.51 (0.37 – 0.68) C: 0.32 (0.16 – 0.46) E: 0.17 (0.14 – 0.20) **Meat and Fish** A: 0.78 (0.63 – 0.92) C: 0.12 (0.00 – 0.27) E: 0.10 (0.08 – 0.12) **Desserts** A: 0.20 (0.04 – 0.38) C: 0.64 (0.46 – 0.77) E: 0.16 (0.12 – 0.22)	Very wide 95% CI

	Foods items	Methods	MZ/DZ	Age	Nationality	Design	Findings	Comments
Food Preference and Sensitivity of Taste for Bitter Compound (Glanville & Kaplan 1965)	• Coffee: with more than 1 spoon of cream/1 spoon of cream/no cream • Coffee: with more than 1 spoon of sugar/1 spoon of sugar/no sugar • Cheese: American/ Longhorn or Swiss/ Blue cheese • Cheddar cheese: mild/medium/sharp • Salad dressing: mild/oil and vinegar/ Blue cheese	A list of five widely used foods/drinks which are commonly prepared in a variety of ways was presented. Subjects were instructed: 'Several common foods are listed below. Each may be prepared in a number of different ways. Please indicate which of the three alternate choices you prefer'	MZ pairs:16 DZ pairs: n=10	38 y Range: 22-66 y	USA	Cross-sectional	MZ preference scores for 'strong and pungent foods' were highly correlated and statically significantly (r=0.7, p<0.01) compared to DZ scores which were low and statistically insignificant (r=0.18)	
Same genetic components underlie different measures of sweet taste preference. (Keskitalo, Tuorila, et al. 2007)	• Sweet foods (sweet desserts, sweets, sweet pastry, ice cream, hard candy, and chocolate)	Self-reported liking of sweet foods 7 point Likert scale	MZ: n=149 pairs DZ: n=175 pairs	55.6 y (12.4) Range: 17 -80 y	UK	Cross-sectional	Sweet food liking: A: 0.54 (0.45 – 0.62) E: 0.46 (0.38 – 0.55)	
Identifying flavour preference subgroups. Genetic basis and related eating behaviour traits (Törnwall et al. 2014)	• 'Basic' food preferences • 'Adventurous' food preferences (higher liking for pungent foods, sour foods, fruits and vegetables)	Specific Food (SF) questionnaire: 13 umami items, 21 sour items and 10 pungent items General Food (GF) questionnaire: 41 Finnish foods 7 point Likert scale	MZ: n=47 pairs DZ: n=93 pairs Unpaired: n=51	22 y Range: 21- 25 y	Finland	Cross-sectional	Genetic and environmental influences underlying the subgroups: A: 0.72 (0.36 – 0.92) E: 0.28 (0.01 – 0.64)	

[1]*Abbreviations: MZ=Monozygotic, DZ= Dizygotic, A=Genetic influences, C=Shared environmental influences, E=Non-shared environmental influences; RMZ= Monozygotic correlation; rDZ= Dizygotic correlation*

Appendix A4 - Twin studies investigating the relative genetic and environmental contributions to drink preferences

	Beverage categories	MZ/DZ	Age	Nationality	Design	Outcomes	Comments
The genetics of tea and coffee drinking and preference for source of caffeine in a large community sample of Australian twins (Luciano et al. 2005)	• How many cups of each tea and coffee they consumed per day? • Overall preference for coffee (cups of coffee consumed / total number of caffeinated drinks consumed)	MZ: n=1796 pairs DZ: n=1092 pairs Unpaired: n=328	34.1 (14.1) Range: 16.3 – 87.3	Australia	Cross-sectional	A: 0.42 (0.32 – 0.46) C: 0.00 (0.00 – 0. 08) E: 0.58 (0.53 – 0.62)	No distinction between regular and decaffeinated tea and coffee was made because at the time of this study decaffeinated products were not common
A Genetic Analysis of Coffee Consumption in a Sample of Dutch Twins (Vink et al. 2009)	• Coffee preference: Number of cups of coffee per day/total number of cups coffee and tea per day	MZ: n=2252 pairs DZ: n=2243pairs Unpaired: n=328	30 (11.3)	Netherlands	Cross-sectional	A: 0.62 (0.44 – 0.79) C: 0.00 (0.00 – 0.00) E: 0.38 (0.26 – 0.55)	

Appendix B.

Chapter 3 - Sampling and Methodology

Appendix B1 Official study participation invitation sent to TEDS twins for the food and drink preference study

TEDS Research Centre
Dept. Box No. P083
Freepost LON7567
London SE5 8YZ

Tel: (0800) 317 029

Email: teds-project@kcl.ac.uk

Fashion, Food and Music Survey
Information Sheet

We would like to invite you to participate in a study about preferences for various fashion styles, foods and music genres. This sheet will provide you with important information about what the study involves. If anything is unclear, or for more information, please contact the TEDS team.

What is the study about?

People vary widely in their preferences for fashions, foods and music, and the origins of these different preferences are often unclear. Conducting this study in TEDS will allow us to explore the sources of these differences and their relationships with other traits.

What does the study involve?

The study consists of a short series of questionnaires. You will be asked for some relevant background information such as your height and weight, and then will be asked to rate your preferences for a variety of different fashion styles, foods, drinks and genres of music. The entire study is conducted online at the TEDS website, and should take around 10-15 minutes to complete.

If you would like to participate, please go to **https://studies.teds.ac.uk/join/prefsqnr** and enter your login ID (from your invitation) when prompted. You will also be asked for your preferred email address, so that we can send you your rewards (see below for details).

If possible, it is best to do the whole study in a single session. If this is not possible or your session is interrupted, you can log in again later, but you may lose some of your progress.

Please don't use your browser's navigation buttons (e.g., Back or Refresh) during the study, as this could cause some of your progress to be lost. If you have any technical problems, please try closing your browser and logging in again, or using a different browser.

The study website is designed for desktop and laptop computers, but should also be compatible with most mobile devices. Some questions involve looking at photos, however, so it would be best to avoid using a device with a small screen.

Rewards

To say thank you for taking part, we will send you (by email) a £10 Love2reward flexecode voucher, which can be used at a wide range of websites (Amazon, iTunes, Lovefilm, etc.). You and your twin will also be entered into a prize draw to win **a pair of iPad Minis** – so your chances of winning are doubled if both you **and** your twin participate! The rewards are only available if you complete the whole study.

The deadline for participating in the study is Sunday 19th April.

Confidentiality

All the information you provide is for research purposes only, and will be kept strictly confidential. TEDS data are sometimes used in future studies and shared with other researchers, but only anonymously, with all personally identifiable information removed. The results of the study will be published in peer-reviewed scientific journals.

It is entirely up to you whether or not to participate. If you choose not to, it will not affect your future involvement with TEDS. If you do participate, you can withdraw again at any time, without having to give a reason.

Queries?

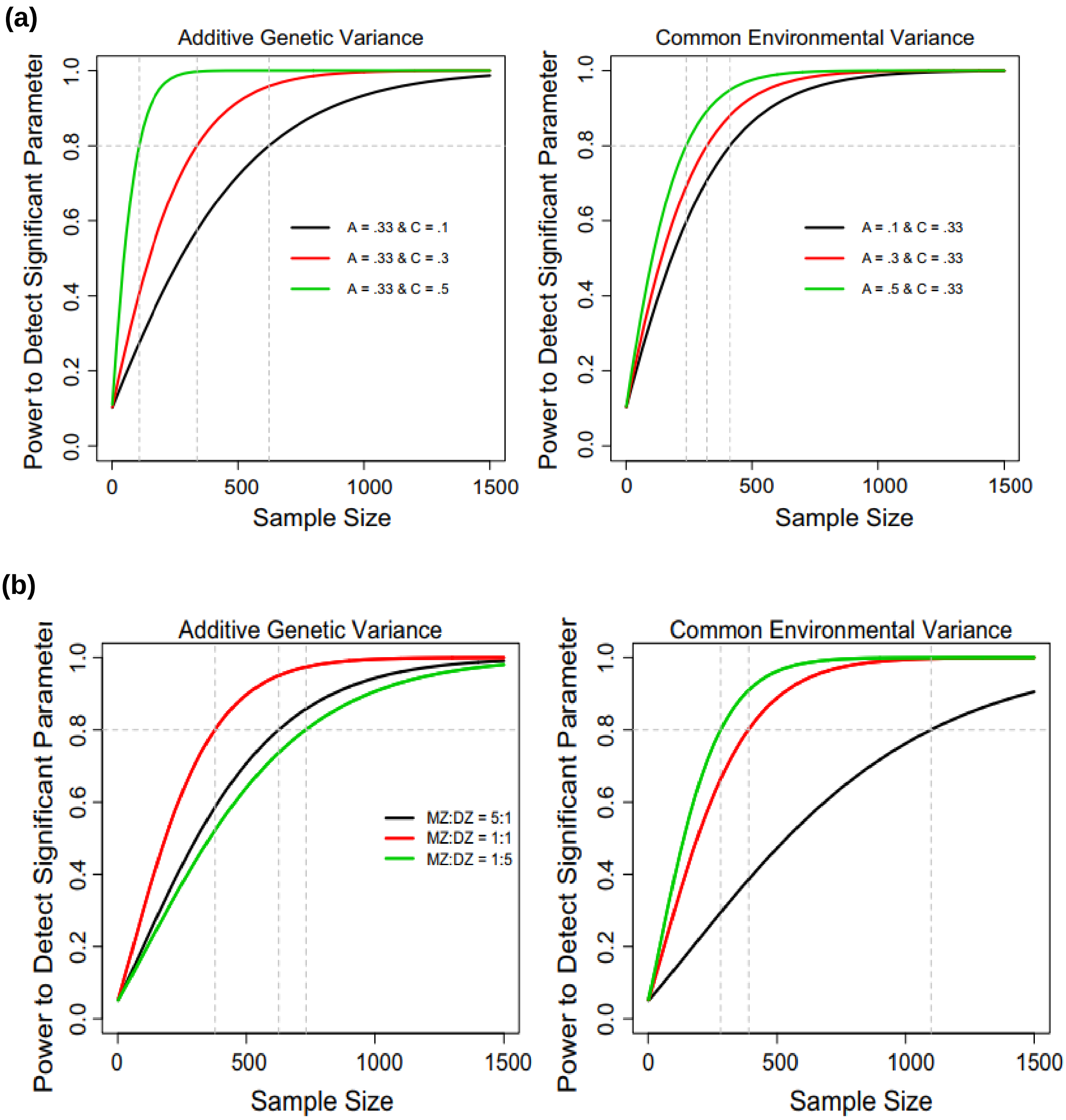
If you have any questions, please do not hesitate to contact us by email, or call our freephone line (details above).

Appendix B2 Summary of 20-item zygosity questionnaire sent to TEDS parents at 1st contact, Year 3 and Year 4

Item	Question	Coding
1	Has a health professional ever told you they are MZ or DZ?	1=MZ, 2=DZ
2	Do you think they are MZ or DZ?	1=MZ, 2=DZ
3	Differences in shade of hair	1=none, 2=slight, 3=clear difference
4	Differences in texture of hair	1=none, 2=slight, 3=clear difference
5	Differences in eye colour	1=none, 2=slight, 3=clear difference
6	Differences in ear-lobe shape	1=none, 2=slight, 3=clear difference
7	Did twins' teeth come through at the same time	1=MZ, 2=DZ
8	Likeness between twins as they became older	1 = greater, 2 = same, 3 = less
9	Can you tell twins apart from a new photo?	1=confuse them, 2=yes, but hard, 3=yes easily
10	Blood group difference (if known)	0=no, 1=yes
11	Blood rhesus factor difference (if known)	0=no, 1=yes
12	Difficulty telling them apart (other parent)	1=often,2=sometimes, 3=rarely/never
13	Difficulty telling them apart (other siblings)	1=often,2=sometimes, 3=rarely/never
14	Difficulty telling them apart (other relatives)	1=often,2=sometimes, 3=rarely/never
15	Difficulty telling them apart (day carer/baby-sitter)	1=often,2=sometimes, 3=rarely/never
16	Difficulty telling them apart (parents' close friends)	1=often,2=sometimes, 3=rarely/never
17	Difficulty telling them apart (parents' casual friends)	1=often,2=sometimes, 3=rarely/never
18	Difficulty telling them apart (people meeting for 1st time)	1=often,2=sometimes, 3=rarely/never
19	Are twins mistaken for each other when together	1=often, 2=sometimes, 3=almost never, 4=never
20	Likeness between the twins	1=alike as two peas in a pod, 2=alike as other sibs, 3=not alike at all

The zygosity algorithm sums all available item scores, divided by the hypothetical maximum score for each individual twin.
Index scores are interpreted as follows: < 0.64 = MZ; 0.64 – 0.70 = indeterminate; ≥ 0.70 = DZ
When the following observations occurred, index scores are disregarded:
Pairs automatically assigned as DZ if parents report clear eye colour differences, hair shade differences, hair texture differences, or that their twins do not look much alike at all
Pairs automatically assigned as MZ if they are said to be as alike as two peas in a pod
Questionnaire adapted from (Price et al. 2000)
Abbreviations: DZ = Dizygotic; MZ = Monozygotic

Figure B3 Power to detect significant parameters in twin studies at (a) different levels of heritability and shared environmental effects, and (b) different levels of MZ:DZ ratios



Figures adapted from Verhulst et al (2017)

Appendix C.

Study 1 - Development of a food and drink preference questionnaire for adolescents and adults

Appendix C1 Food and drink preference questionnaire disseminated to TEDS participants

Food and drink preference survey

[Standard wording for TEDS briefing / consent, describing the study as a "brief questionnaire about your fashion, food and music preferences" taking around 10-15 minutes to complete. Include an information sheet, consent page and validated email address form.]

[Where appropriate, value coding is given here in square brackets. Multiple-choice questions are coded as integers, typically 1-n, but with unambiguously negative responses ('No', 'Not at all', 'Not applicable', etc.) coded 0.]

Background information

1. What is your height?

Centimetres *[text box with validation: numbers only, range 75-250]*
OR Feet and inches *[drop-down, 1-7', 1-11"]*
OR Inches *[text box with validation: numbers only, range 30-100]*

2. What is your current weight (approximately)?

Kilograms *[text box with validation: numbers only, range 18-180]*
OR Stones and pounds *[drop-down: 1-22 st, 1-13 lb]*
OR Pounds *[text box with validation: numbers only, range 40-400]*

3. How would you describe your current weight?

[radio]
Very underweight [1]
Underweight [2]
About the right weight [3]
Somewhat overweight [4]
Very overweight [5]
Obese [6]

Section 2: Food preferences

This section is about your likes and dislikes for various foods and drinks. We are not trying to assess how healthy or unhealthy your food preferences are, so no need to worry; there are no right or wrong answers!

15. In general, how healthy would you say your overall diet is?

[radio]
Very healthy [5]
Somewhat healthy [4]
Neither particularly healthy nor unhealthy [3]
Somewhat unhealthy [2]
Very unhealthy [1]

16. Do you identify as any of the following?

[radio]
Vegan [3]
Vegetarian [2]
Pescetarian (no meat, but eat fish and/or shellfish) [1]
None of the above [0]

17. Are you allergic to any of the following food items? (please select all that apply)

[checkbox; values coded 1=true, 0=false]
Peanuts
Tree nuts
Sesame
Dairy
Shellfish
Fish
Egg
Wheat / Gluten
Soya
Celery
Mustard
Other (please specify) *[branch: enable text input, 30 char limit]*

Food preference ratings

Briefly read the following list of food/drink items and tick the box which most accurately reflects how much (on average) you like the specific item (not necessarily how much you actually consume).

For any foods you don't know, or don't remember ever having tried, please select "Not applicable".

[tabulated radio questions, each with options: "Dislike a lot", "Dislike a little", "Neither like nor dislike", "Like a little", "Like a lot" and "Not applicable".]
[Values coded: 1, 2, 3, 4, 5, 0]

[Table] **How much do you like…**

18. Marmite (or Vegemite)?

19. Beef?

20. Beef burgers?

21. Lamb?

22. Chicken?

23. Bacon?

24. Ham?

25. Liver (e.g., pate, liver sausage)?

26. Sausages?

27. White fish (e.g., cod, haddock)?

28. Oily fish (e.g., mackerel, kippers)?

29. Smoked salmon?

30. Tinned tuna?

31. Eggs (boiled, scrambled or fried)?

32. Baked beans?

33. Nuts (e.g., almonds, brazil nuts)?

34. Bread or bread rolls?

35. Bran cereal (e.g., All Bran, Bran Flakes)?

36. Porridge?

Food preference ratings

For any foods you don't know, or don't remember ever having tried, please select "Not applicable".

[tabulated radio questions, each with options: "Dislike a lot", "Dislike a little", "Neither like nor dislike", "Like a little", "Like a lot" and "Not applicable".]
[Values coded: 1, 2, 3, 4, 5, 0]

[Table] **How much do you like...**

37. Plain boiled rice?

38. Sugared cereal (e.g., Frosties, Sugar Puffs)?

39. Hummus?

40. Wheat cereal (e.g., Weetabix, Shredded Wheat)?

41. Potatoes (boiled or mashed)?

42. Chips?

43. Rice or corn cereal (e.g., Corn Flakes, Rice Krispies)?

44. Soft cheese (e.g., Camembert, Brie)?

45. Hard cheese (e.g., Cheddar)?

46. Cottage cheese?

47. Plain, low-fat yoghurt?

48. Oranges?

49. Grapes?

50. Apples?

51. Melon?

52. Peaches?

53. Apricots?

54. Strawberries?

55. Avocado?

Food preference ratings

For any foods you don't know, or don't remember ever having tried, please select "Not applicable".

[tabulated radio questions, each with options: "Dislike a lot", "Dislike a little", "Neither like nor dislike", "Like a little", "Like a lot" and "Not applicable".]
[Values coded: 1, 2, 3, 4, 5, 0]

[Table] **How much do you like…**

56. Spinach?

57. Carrots?

58. Green beans?

59. Cucumber?

60. Celery?

61. Mushrooms?

62. Parsnips?

63. Peas?

64. Sweetcorn?

65. Broccoli?

66. Salad leaves (e.g., lettuce)?

67. Red peppers?

68. Raw tomatoes?

69. Beetroot?

70. Brussel sprouts?

71. Vegetable soup?

72. Coriander (the green herb also referred to as cilantro)?

Food preference ratings

For any foods you don't know, or don't remember ever having tried, please select "Not applicable".

[tabulated radio questions, each with options: "Dislike a lot", "Dislike a little", "Neither like nor dislike", "Like a little", "Like a lot" and "Not applicable".]
[Values coded: 1, 2, 3, 4, 5, 0]

[Table] **How much do you like...**

73. Butter?

74. Butter-like spreads (e.g., Sunflower spread, Flora)?

75. Cream?

76. Mayonnaise?

77. Plain biscuits (e.g., digestives)?

78. Chocolate biscuits?

79. Cake?

80. Apple pie?

81. Ice cream?

82. Custard?

83. Chocolate?

84. Crisps?

85. Peanut butter?

86. Chewy gummy sweets (e.g., Haribo-style sweets, Wine gums)?

Drink preference ratings

87. What type of milk do you drink?

[radio]
Full-fat milk [4]
Semi-skimmed milk [3]
Skimmed milk [2]
Non-dairy milk (e.g. soy milk, almond milk) [1]
I don't drink milk [0]

88. How much do you like milk?

[radio. Branch: disabled until Q87 response, then enabled if Q87!="I don't drink milk"]
Dislike a lot [1]
Dislike a little [2]
Neither like nor dislike [3]
Like a little [4]
Like a lot [5]

89. Do you drink (or have you ever drunk) coffee?

Yes [1]
No [0]

90. How much do you like coffee?

[radio. Branch: disabled until Q89 response, then enabled if Q89="Yes"]
Dislike a lot [1]
Dislike a little [2]
Neither like nor dislike [3]
Like a little [4]
Like a lot [5]

[tabulated radio questions. Branch: disabled until Q89 response, then enabled if Q89="Yes"]

How do you drink coffee?

91. Black or white? [Black [0]/ White [1]]

92. Unsweetened or sweetened? [Unsweetened [0] / Sweetened [1]]

93. Do you drink (or have you ever drunk) tea?

[radio]
Yes [1]
No [0]

94. How much do you like tea?

[radio. Branch: disabled until Q93 response, then enabled if Q93="Yes"]
Dislike a lot [1]
Dislike a little [2]
Neither like nor dislike [3]
Like a little [4]
Like a lot [5]

[tabulated radio questions. Branch: disabled until Q93 response, then enabled if Q93="Yes"]

How do you drink tea?

95. Black or white? [Black [0]/ White [1]]

96. Unsweetened or sweetened? [Unsweetened [0] / Sweetened [1]]

Drink preference ratings

For any drinks you don't know, or don't remember ever having tried, please select "Not applicable".

[tabulated radio questions, each with options: "Dislike a lot", "Dislike a little", "Neither like nor dislike", "Like a little", "Like a lot" and "Not applicable".]
[Values coded: 1, 2, 3, 4, 5, 0]

[Table] **How much do you like...**

97. Non-diet fizzy drinks (e.g., Coca Cola, Pepsi)?

98. Diet fizzy drinks (e.g., Diet Cola, Pepsi Max)?

99. Orange juice?

100. Ribena or other fruit squash (e.g., orange squash)?

101. Which type of wine do you prefer?

[radio]
White wine [5]
Red wine [4]
Rosé wine [3]
Like all [2]
Don't like any [1]
I don't drink wine [0]

102. How much do you like wine?

[radio. Branch: disabled until Q101 response, then enabled if Q101!="I don't drink wine"]
Dislike a lot [1]
Dislike a little [2]
Neither like nor dislike [3]
Like a little [4]
Like a lot [5]

103. How much do you like beer?

[radio]
Dislike a lot [1]
Dislike a little [2]
Neither like or dislike [3]
Like a little [4]
Like a lot [5]
Not applicable [0]

Food preference ratings

104. How do you prefer popcorn?
[radio]
Sweet [4]
Salty [3]
Like both [2]
I don't like either [1]
Not applicable [0]

Having a "sweet tooth" describes an individual with a fondness of sweet things, and a tendency to crave sweet treats rather than savoury snacks.

105. Would you say you have a "sweet tooth"?
[radio]
Not at all [0]
Not really [1]
Neither agree nor disagree [2]
Somewhat [3]
Very much [4]

106. How much do you like spicy (hot) food?
[radio]
Dislike a lot [1]
Dislike a little [2]
Neither like or dislike [3]
Like a little [4]
Like a lot [5]
Not applicable [0]

107. How frequently do you add salt to your food?
[radio]
Never [0]
Rarely [1]
Sometimes [2]
Usually [3]
Always [4]

108. Please leave a comment on what you thought about filling in this questionnaire (e.g. level of engagement, clarity of questions, and structure of layout).
[textarea, max 500 characters]

109. Is there anything else you would like to mention in relation to the topics covered that you feel was not adequately addressed in the questions?

[textarea, max 500 characters]

Appendix D.

Study 2 - Genetic and environmental influences on food and drink preferences

Appendix D1 Poster on the aetiology of non-alcoholic beverage preferences (presented at The Obesity Society's Annual Scientific Meeting [2016] New Orleans, USA)

Genetic and environmental etiology of non-alcoholic drink preferences in adolescence

Andrea Smith[1], Alison Fildes[1], Lucy Cooke[1], Nicholas Shakeshaft[2], Robert Plomin[2] and Clare Llewellyn[1]

[1] *Health Behaviour Research Centre, Department of Epidemiology and Public Health, UCL, UK*
[2] *Social, Genetic & Developmental Psychiatry Centre, Kings College London, UK*

UCL

Why are drink preferences important?

- **Drink preferences drive actual drink intake.**
- Consumption of too many sugar-sweetened beverages (SSBs) or juice drinks **increases the risk of a number of chronic diseases**, including: type 2 diabetes[1], heart disease[2] and liver disease[3].
- Understanding the etiology of variation in drink preferences is necessary for the **development of drink (sweetness) preference modification interventions.**
- **Adolescence** is the transition into adulthood, characterised by increasing autonomy over eating and drinking behavior.

Background

Twin studies can estimate the relative contribution of genetic and environmental influences to variation in any given trait, such as drink preferences[4].

Variation is apportioned to:

- **Additive genetic influence**, so-called *heritability*: an aggregate of total genomic variation.
- **Shared environmental influence**: aspects of the environmental completely shared by twin pairs, e.g. socioeconomic status.
- **Non-shared environmental influence**: aspects of the environment not shared by twin pairs, e.g. illness.

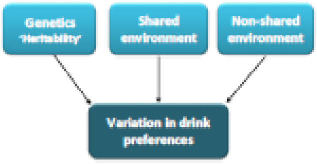
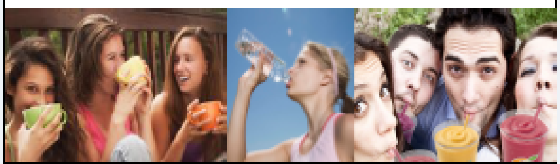


Figure 1. Conceptual framework of the three main domains that influence individual drink preferences

Only 2 previous studies have explored the heritability of drink preferences. Liking for sweet solutions was influenced by moderate genetic factors (27-41%), and variation in these preferences were associated with a locus on chromosome 16 (*16p11.2*)[5].

Moderate heritability of high-sugar drink intake (48%) has been demonstrated in adults, with the non-shared environment accounting for the remaining variance. This suggests that individual lifestyle factors are the main environmental shapers of drink preferences in adulthood[7].



Aim

To estimate for the first time the relative contribution of genetic and environmental influences on a range of non-alcoholic drink preferences in adolescence.

Data and Methods

Sample: 2865 twins (18-19 years old) from the Twins Early Development Study (TEDS), a large population-based cohort of British twins born in 1994-6[8].

Drink preferences: Liking for 5 drink types (sugar-sweetened-beverages [SSBs]; non-nutritive sweetened beverages [NNBs]; fruit cordials, orange juice and milk) was assessed by questionnaire, using a 5-point Likert scale ('Not at all' to 'A lot').

Zygosity: 20-item questionnaire, shown to be 96% reliable using DNA tests[9].

Twin analyses:

(1) Intraclass correlations (ICC) were calculated for each zygosity group [monozygotic (MZ; identical twins) and dizygotic (DZ; non-identical twins)] to establish within-pair resemblance for drink preferences. Greater MZ than DZ resemblance indicates a greater genetic contribution to variation.

(2) Maximum Likelihood structural equation modelling (MLSEM) was used to estimate genetic (A), shared environmental (C), and unique environmental effects (E)

Results

1) Intraclass correlations

For all five drinks, MZ correlations were higher than DZ correlations; implicating a genetic influence on drink preferences.

Table 1. Drink preference scores and ICC's by zygosity

	n	Mean preference score (SD)[a]	MZ ICC[b]	DZ ICC[b]
Fruit squash	2847	4.23 (1.02)	0.422	0.212
NNSBs[b]	2827	3.64 (1.34)	0.393	0.222
SSBs[b]	2841	3.73 (1.37)	0.383	0.155
Milk	2707	4.22 (0.95)	0.377	0.091
Orange juice	2849	4.43 (0.97)	0.261	0.000

[a] Preference scores were rated on a 5 point Likert scale, with a higher score indicating a higher preference
[b] Abbreviations: ICCs: Intraclass Correlations; MZ: Monozygotic; DZ: Dizygotic; SSB: Sugar-sweetened beverage; NNSB: Non-nutritive sweetened beverage

(2) Maximum Likelihood structural equation modelling

Genetic effects were low to moderate for all drink types.

No shared-environment effects on any drink preferences.

Non-shared environment effects explained remaining variation for all drink preferences.

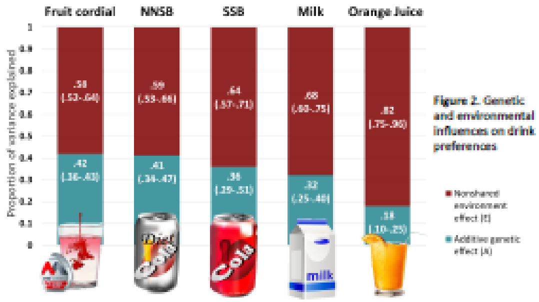


Figure 2. Genetic and environmental influences on drink preferences

Strengths and Limitations

Limitations

- Influences can change over the lifespan; results may not generalise to other ages
- Limited range of drink types; a more detailed questionnaire (incl. energy drinks, smoothies) may be useful for future research

Strengths

- First study to establish the etiology of drink preferences in adolescents
- Large sample

Summary and Conclusions

- Individual differences in drink preferences have a moderate genetic basis in adolescence, whereas the shared environment exerts no detectable effect.
- The unique environment exerts a sizeable influence on the drink preferences of older adolescents
- Efforts to modify drink preferences of older adolescents may be best targeted at the wider environment e.g. government policies to discourage consumption of unhealthy drinks and to support access and affordability of healthier drink options[10].

References

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MRC Social, Genetic and Developmental Psychiatry Centre

TEDS

Appendix D2 Model fit and parameter estimates for the saturated, ACE model and submodels of 69 food item preference scores

Food item	Additive genetic effect (A)	Shared environment effect (C)	Nonshared environment effect (E)	-2LL[3]	Df[3]	AIC[3]
Beef						
Sat				7452.189	2621	2176.189
ACE[1]	0.41 (0.33, 0.48)	0.00 (0.00, 0.08)	0.59 (0.52, 0.67)	7464.075	2641	2182.075
AE[2]	**0.41 (0.33, 0.48)**	**-**	**0.59 (0.52, 0.67)**	**7464.075**	**2642**	**2180.075**
CE[2]	-	0.25 (0.19, 0.31)	0.75 (0.69, 0.81)	7492.904	2642	2208.904
E	-	-	1.00 (1.00, 1.00)	7605.823	2626	2319.823
Beef burgers						
Sat				7253.788	2614	2025.788
ACE[1]	0.32 (0.25, 0.40)	0.00 (0.00, 0.10)	0.68 (0.60, 0.70)	7263.180	2617	2029.180
AE[2]	**0.32 (0.32, 0.40)**	**-**	**0.68 (0.60, 0.68)**	**7263.180**	**2618**	**2027.180**
CE[2]	-	0.20 (0.14, 0.26)	0.80 (0.74, 0.86)	7274.044	2618	2038.044
E	-	-	1.00 (1.00, 1.00)	7311.635	2619	2073.635
Lamb						
Sat				8554.181	2601	3352.181
ACE[1]	0.51 (0.34, 0.57)	0.00 (0.00, 0.13)	0.49 (0.43, 0.56)	8555.542	2604	3347.542
AE[2]	**0.51 (0.44, 0.57)**	**-**	**0.49 (0.43, 0.56)**	**8555.542**	**2605**	**3345.542**
CE[2]	-	0.34 (0.28, 0.39)	0.66 (0.61, 0.72)	8581.270	2605	3371.270
E	-	-	1.00 (1.00, 1.00)	8702.839	2606	3490.839
Chicken						
Sat				3975.058	2635	-1294.942
ACE[1]	0.19 (0.00, 0.28)	0.00 (0.00, 0.16)	0.81 (0.72, 0.89)	3985.977	2638	-1290.023
AE[2]	**0.19 (0.13, 0.28)**	**-**	**0.81 (0.72, 0.87)**	**3985.977**	**2639**	**-1292.023**
CE[2]	-	0.13 (0.06, 0.19)	0.87 (0.81, 0.94)	3988.849	2639	-1289.151
E	-	-	1.00 (1.00, 1.00)	4003.843	2640	-1276.157
Bacon						
Sat				7088.937	2598	1892.937
ACE[1]	0.28 (0.23, 0.36)	0.00 (0.00, 0.06)	0.72 (0.64, 0.78)	7127.746	2601	1925.746
AE[2]	**0.28 (0.22, 0.36)**	**-**	**0.72 (0.64, 0.78)**	**7127.746**	**2602**	**1923.746**
CE[2]	-	0.16 (0.10, 0.22)	0.84 (0.78, 0.90)	7142.572	2602	1938.572
E	-	-	1.00 (1.00, 1.00)	7167.404	2603	1961.404
Ham						
Sat				7550.676	2599	2352.676
ACE[1]	0.34 (0.24, 0.42)	0.00 (0.00, 0.08)	0.66 (0.58, 0.74)	7553.863	2602	2349.863
AE[2]	**0.34 (0.26, 0.42)**	**-**	**0.66 (0.58, 0.74)**	**7553.863**	**2603**	**2347.863**
CE[2]	-	0.21 (0.15 0.27)	0.79 (0.73, 0.85)	7570.332	2603	2364.332
E	-	-	1.00 (1.00, 1.00)	7615.496	2604	2407.496
Liver						
Sat				7985.341	2343	3299.341
ACE[1]	0.38 (0.17, 0.52)	0.07 (0.00, 0.24)	0.55 (0.48, 0.64)	7985.803	2346	3293.803
AE[2]	**0.46 (0.38, 0.52)**	**-**	**0.54 (0.48, 0.62)**	**7986.349**	**2347**	**3292.349**
CE[2]	-	0.33 (0.27, 0.38)	0.67 (0.62, 0.73)	7996.560	2347	3302.560
E	-	-	1.00 (1.00, 1.00)	8096.482	2348	3400.482

Food item	Additive genetic effect (A)	Shared environment effect (C)	Nonshared environment effect (E)	-2LL[3]	Df[3]	AIC[3]
Sausages						
Sat				7158.807	2623	1912.807
ACE[1]	0.29 (0.18, 0.36)	0.00 (0.00, 0.06)	0.71 (0.64, 0.80)	7168.769	2626	1916.769
AE[2]	**0.29 (0.20, 0.36)**	**-**	**0.71 (0.64, 0.80)**	**7168.769**	**2627**	**1914.769**
CE[2]	-	0.17 (0.11, 0.23)	0.83 (0.77, 0.89)	7183.650	2627	1929.650
E	-	-	1.00 (1.00, 1.00)	7212.060	2628	1956.060
White fish						
Sat				8959.936	2691	3577.936
ACE[1]	0.34 (0.23, 0.41)	0.00 (0.00, 0.08)	0.66 (0.59, 0.74)	8971.942	2694	3583.942
AE[2]	**0.34 (0.26, 0.41)**	**-**	**0.66 (0.59, 0.74)**	**8971.942**	**2695**	**3581.942**
CE[2]	-	0.21 (0.15, 0.27)	0.79 (0.73, 0.85)	8989.200	2695	3599.200
E	-	-	1.00 (1.00, 1.00)	9033.962	2696	3641.962
Oily fish						
Sat				9144.374	2546	4052.374
ACE[1]	0.52 (0.44, 0.58)	0.00 (0.00, 0.05)	0.48 (0.42, 0.55)	9151.265	2549	4053.265
AE[2]	**0.52 (0.45, 0.58)**	**-**	**0.48 (0.42, 0.55)**	**9151.265**	**2550**	**4051.265**
CE[2]	-	0.32 (0.26, 0.37)	0.68 (0.63, 0.74)	9194.508	2550	4094.508
E[2]	-	-	1.00 (1.00, 1.00)	9299.305	2551	4197.305
Smoked salmon						
Sat				9795.777	2599	4597.777
ACE[1]	0.40 (0.26, 0.47)	0.03 (0.00, 0.10)	0.60 (0.53, 0.67)	9796.760	2602	4592.760
AE[2]	**0.40 (0.33, 0.47)**	**-**	**0.60 (0.53, 0.67)**	**9796.760**	**2603**	**4590.760**
CE[2]	-	0.26 (0.21, 0.32)	0.74 (0.68, 0.79)	9814.676	2603	4608.676
E	-	-	1.00 (1.00, 1.00)	9887.440	2604	4679.440
Tinned tuna						
Sat				9761.199	2671	4419.199
ACE[1]	0.54 (0.44, 0.60)	0.00 (0.00, 0.08)	0.46 (0.40, 0.53)	9764.078	2674	4416.078
AE[2]	**0.54 (0.47, 0.60)**	**-**	**0.46 (0.40, 0.53)**	**9764.078**	**2675**	**4414.078**
CE[2]	-	0.35 (0.29, 0.40)	0.65 (0.60, 0.71)	9804.289	2675	4454.289
E	-	-	1.00 (1.00, 1.00)	9938.552	2676	4586.552
Eggs						
Sat				9174.151	2813	3548.151
ACE[1]	0.33 (0.25, 0.40)	0.00 (0.00, 0.03)	0.67 (0.60, 0.75)	9195.705	2816	3563.705
AE[2]	**0.33 (0.25, 0.40)**	**-**	**0.67 (0.60, 0.75)**	**9195.705**	**2817**	**3561.705**
CE[2]	-	0.18 (0.12, 0.23)	0.82 (0.77, 0.88)	9225.110	2817	3591.110
E	-	-	1.00 (1.00, 1.00)	9261.607	2818	3625.607
Baked beans						
Sat				9370.724	2835	3700.724
ACE[1]	0.28 (0.18, 0.36)	0.00 (0.00, 0.06)	0.72 (0.64, 0.79)	9381.408	2838	3705.408
AE[2]	**0.28 (0.21, 0.36)**	**-**	**0.72 (0.64, 0.79)**	**9381.408**	**2839**	**3703.408**
CE[2]	-	0.16 (0.11, 0.22)	0.84 (0.78, 0.89)	9396.345	2839	3718.345
E	-	-	1.00 (1.00, 1.00)	9428.554	2840	3748.554
Nuts						
Sat				9648.361	2801	4046.361
ACE[1]	0.39 (0.26, 0.46)	0.00 (0.00, 0.09)	0.61 (0.54, 0.68)	9650.407	2804	4042.407
AE[2]	**0.39 (0.32, 0.46)**	**-**	**0.61 (0.54, 0.68)**	**9650.407**	**2805**	**4040.407**
CE[2]	-	0.25 (0.20, 0.31)	0.75 (0.69, 0.80)	9670.037	2805	4060.037
E	-	-	1.00 (1.00, 1.00)	9746.416	2806	4134.416

Food item	Additive genetic effect (A)	Shared environment effect (C)	Nonshared environment effect (E)	-2LL[3]	Df[3]	AIC[3]
Bread						
Sat				6355.631	2850	655.6312
ACE[1]	0.18 (0.00, 0.25)	0.00 (0.00, 0.12)	0.82 (0.75, 0.90)	6362.331	2853	656.3309
AE[2]	**0.18 (0.10, 0.25)**	**-**	**0.82 (0.75, 0.90)**	**6362.331**	**2854**	**654.3309**
CE[2]	-	0.12 (0.06, 0.17)	0.88 (0.83, 0.94)	6366.269	2854	658.2686
E	-	-	1.00 (1.00, 1.00)	6382.106	2855	672.1059
Bran cereal						
Sat				9040.814	2781	3478.814
ACE[1]	0.35 (0.26, 0.42)	0.00 (0.00, 0.06)	0.65 (0.58, 0.72)	9047.336	2784	3479.336
AE[2]	**0.35 (0.28, 0.42)**	**-**	**0.65 (0.58, 0.72)**	**9047.336**	**2785**	**3477.336**
CE[2]	-	0.21 (0.16, 0.27)	0.79 (0.73, 0.84)	9069.445	2785	3499.445
E	-	-	1.00 (1.00, 1.00)	9123.411	2786	3551.411
Porridge						
Sat				9610.687	2802	4006.687
ACE[1]	0.39 (0.32, 0.46)	0.00 (0.00, 0.04)	0.61 (0.54, 0.68)	9622.335	2805	4012.335
AE[2]	**0.39 (0.32, 0.46)**	**-**	**0.61 (0.54, 0.68)**	**9622.335**	**2806**	**4010.335**
CE[2]	-	0.23 (0.18, 0.28)	0.77 (0.72, 0.82)	9655.437	2806	4043.437
E[2]	-	-	1.00 (1.00, 1.00)	9721.285	2807	4107.285
Rice						
Sat				8255.540	2842	2571.540
ACE[1]	0.27 (0.15, 0.34)	0.00 (0.00, 0.08)	0.73 (0.66, 0.81)	8259.105	2845	2569.105
AE[2]	**0.27 (0.19, 0.34)**	**-**	**0.73 (0.66, 0.81)**	**8259.105**	**2846**	**2567.105**
CE[2]	-	0.17 (0.11, 0.22)	0.83 (0.78, 0.89)	8271.218	2846	2579.218
E	-	-	1.00 (1.00, 1.00)	8305.750	2847	2611.750
Sugared cereal						
Sat				8650.660	2842	2966.660
ACE[1]	0.32 (0.14, 0.43)	0.04 (0.00, 0.20)	0.64 (0.57, 0.73)	8654.117	2845	2964.117
AE[2]	**0.37 (0.30, 0.44)**	**-**	**0.63 (0.56, 0.70)**	**8654.418**	**2846**	**2962.418**
CE[2]	-	0.26 (0.20, 0.31)	0.74 (0.69, 0.80)	8662.606	2846	2970.606
E	-	-	1.00 (1.00, 1.00)	8743.169	2847	3049.169
Hummus						
Sat				9121.570	2488	4145.570
ACE[1]	0.52 (0.32, 0.59)	0.00 (0.00, 0.16)	0.48 (0.41, 0.59)	9123.157	2491	4141.157
AE[2]	**0.52 (0.46, 0.59)**	**-**	**0.48 (0.41, 0.54)**	**9123.158**	**2492**	**4139.158**
CE[2]	-	0.36 (0.30, 0.41)	0.64 (0.59, 0.70)	9146.882	2492	4162.882
E	-	-	1.00 (1.00, 1.00)	9273.866	2493	4287.866
Wheat cereal						
Sat				8397.267	2807	2783.267
ACE[1]	0.29 (0.17, 0.36)	0.00 (0.00, 0.08)	0.71 (0.64, 0.79)	8408.945	2810	2788.945
AE[2]	**0.29 (0.21, 0.36)**	**-**	**0.71 (0.64, 0.79)**	**8408.945**	**2811**	**2786.945**
CE[2]	-	0.18 (0.12, 0.23)	0.82 (0.77, 0.88)	8421.581	2811	2799.581
E	-	-	1.00 (1.00, 1.00)	8458.400	2812	2834.400
Potatoes						
Sat				7717.038	2851	2015.038
ACE[1]	0.27 (0.20, 0.34)	0.00 (0.00, 0.06)	0.73 (0.66, 0.70)	7723.522	2854	2015.522
AE[2]	**0.27 (0.20, 0.34)**	**-**	**0.73 (0.66, 0.80)**	**7723.522**	**2855**	**2013.522**
CE[2]	-	0.16 (0.11, 0.21)	0.84 (0.79, 0.89)	7739.159	2855	2029.159
E	-	-	1.00 (1.00, 1.00)	7772.137	2856	2060.137

Food item	Additive genetic effect (A)	Shared environment effect (C)	Nonshared environment effect (E)	-2LL[3]	Df[3]	AIC[3]
Apples						
Sat				7783.762	2849	2085.762
ACE[1]	0.20 (0.03, 0.27)	0.00 (0.00, 0.13)	0.80 (0.73, 0.87)	6763.066	2852	1059.066
AE[2]	**0.20 (0.13, 0.27)**	**-**	**0.80 (0.73, 0.87)**	**6763.066**	**2853**	**1057.066**
CE[2]	-	0.14 (0.08, 0.19)	0.86 (0.81, 0.92)	6768.153	2853	1062.153
E	-	-	1.00 (1.00, 1.00)	6791.986	2854	1083.986
Melons						
Sat				9429.230	2830	3769.230
ACE[1]	0.33 (0.23, 0.40)	0.00 (0.00, 0.10)	0.67 (0.60, 0.74)	9434.591	2833	3768.591
AE[2]	**0.33 (0.26, 0.40)**	**-**	**0.67 (0.60, 0.74)**	**9434.591**	**2834**	**3766.591**
CE[2]	-	0.22 (0.16, 0.27)	0.78 (0.73, 0.84)	9448.145	2834	3780.145
E	-	-	1.00 (1.00, 1.00)	9503.328	2835	3833.328
Peaches						
Sat				8992.664	2786	3420.664
ACE[1]	0.47 (0.29, 0.53)	0.00 (0.00, 0.13)	0.53 (0.47, 0.59)	8995.954	2789	3417.954
AE[2]	**0.47 (0.41, 0.53)**	**-**	**0.53 (0.47, 0.59)**	**8995.954**	**2790**	**3415.954**
CE[2]	-	0.33 (0.28, 0.38)	0.67 (0.62, 0.72)	9019.375	2790	3439.375
E[2]	-	-	1.00 (1.00, 1.00)	9154.307	2791	3572.307
Apricots						
Sat				9357.675	2727	3903.675
ACE[1]	0.33 (0.12, 0.45)	0.04 (0.00, 0.20)	0.53 (0.55, 0.71)	9359.108	2730	3899.108
AE[2]	**0.38 (0.31, 0.45)**	**-**	**0.62 (0.55, 0.69)**	**9359.391**	**2731**	**3897.391**
CE[2]	-	0.27 (0.22, 0.33)	0.73 (0.67, 0.78)	9368.312	2731	3906.312
E	-	-	1.00 (1.00, 1.00)	9454.140	2732	3990.140
Strawberries						
Sat				7818.806	2841	2136.806
ACE[1]	0.40 (0.33, 0.47)	0.00 (0.00, 0.03)	0.60 (0.53, 0.67)	7840.253	2844	2152.253
AE[2]	**0.40 (0.33, 0.47)**	**-**	**0.60 (0.53, 0.67)**	**7840.253**	**2845**	**2150.253**
CE[2]	-	0.22 (0.16, 0.27)	0.78 (0.73, 0.84)	7879.864	2845	2189.864
E	-	-	1.00 (1.00, 1.00)	7940.792	2846	2248.792
Avocado						
Sat				8791.319	2446	3899.319
ACE[1]	0.53 (0.41, 0.59)	0.00 (0.00, 0.09)	0.47 (0.41, 0.54)	8793.437	2449	3895.437
AE[2]	**0.53 (0.46, 0.59)**	**-**	**0.47 (0.41, 0.54)**	**8793.437**	**2450**	**3893.437**
CE[2]	-	0.35 (0.29, 0.40)	0.65 (0.60, 0.71)	8827.847	2450	3927.847
E	-	-	1.00 (1.00, 1.00)	8949.942	2451	4047.942
Spinach						
Sat				9375.947	2677	4021.947
ACE[1]	0.45 (0.38, 0.52)	0.00 (0.00, 0.08)	0.55 (0.48, 0.62)	9378.450	2680	4018.450
AE[2]	**0.45 (0.38, 0.52)**	**-**	**0.55 (0.48, 0.62)**	**9378.450**	**2681**	**4016.450**
CE[2]	-	0.28 (0.23, 0.34)	0.72 (0.66, 0.77)	9405.344	2681	4043.344
E	-	-	1.00 (1.00, 1.00)	9491.797	2682	4127.797

Food item	Additive genetic effect (A)	Shared environment effect (C)	Nonshared environment effect (E)	-2LL[3]	Df[3]	AIC[3]
Chips						
Sat				6456.829	2852	752.8289
ACE[1]	0.29 (0.20, 0.36)	0.00 (0.00, 0.05)	0.71 (0.64, 0.79)	6464.569	2855	754.5689
AE[2]	**0.29 (0.21, 0.36)**	**-**	**0.71 (0.64, 0.79)**	**6464.569**	**2856**	**752.5689**
CE[2]	-	0.17 (0.11, 0.22)	0.83 (0.78, 0.89)	6481.715	2856	769.7153
E	-	-	1.00 (1.00, 1.00)	6515.678	2857	801.6778
Rice/Corn cereal						
Sat				8017.177	2845	2327.177
ACE[1]	0.27 (0.19, 0.35)	0.00 (0.00, 0.08)	0.73 (0.65, 0.81)	8026.405	2848	2330.405
AE[2]	**0.27 (0.21, 0.35)**	**-**	**0.73 (0.65, 0.79)**	**8026.405**	**2849**	**2328.405**
CE[2]	-	0.16 (0.16, 0.22)	0.84 (0.78, 0.84)	8038.089	2849	2340.089
E	-	-	1.00 (1.00, 1.00)	8068.982	2850	2368.982
Soft Cheese						
Sat				9665.662	2716	4233.662
ACE[1]	0.46 (0.32, 0.52)	0.00 (0.00, 0.11)	0.54 (0.48, 0.60)	9667.726	2719	4229.726
AE[2]	**0.46 (0.40, 0.52)**	**-**	**0.54 (0.48, 0.60)**	**9667.726**	**2720**	**4227.726**
CE[2]	-	0.31 (0.26, 0.36)	0.69 (0.64, 0.74)	9692.689	2720	4252.689
E[2]	-	-	1.00 (1.00, 1.00)	9803.590	2721	4361.590
Hard cheese						
Sat				8538.900	2794	2950.900
ACE[1]	0.25 (0.14, 0.33)	0.00 (0.00, 0.07)	0.75 (0.67, 0.83)	8544.294	2797	2950.294
AE[2]	**0.25 (0.17, 0.33)**	**-**	**0.75 (0.67, 0.83)**	**8544.294**	**2798**	**2948.294**
CE[2]	-	0.15 (0.09, 0.21)	0.85 (0.79, 0.91)	8555.405	2798	2959.405
E	-	-	1.00 (1.00, 1.00)	8579.466	2799	2981.466
Cottage cheese						
	Sat			8724.676	2516	3692.676
ACE[1]	0.39 (0.31, 0.46)	0.00 (0.00, 0.13)	0.61 (0.54, 0.69)	8724.938	2519	3686.938
AE[2]	**0.39 (0.31, 0.46)**	**-**	**0.61 (0.54, 0.69)**	**8724.938**	**2520**	**3684.938**
CE[2]	-	0.26 (0.20, 0.32)	0.74 (0.68, 0.80)	8738.321	2520	3698.321
E[2]	-	-	1.00 (1.00, 1.00)	8808.279	2521	3766.279
Yoghurt						
Sat				8853.704	2749	3355.704
ACE[1]	0.26 (0.15, 0.34)	0.00 (0.00, 0.07)	0.74 (0.66, 0.81)	8858.956	2752	3354.956
AE[2]	**0.26 (0.19, 0.34)**	**-**	**0.74 (0.66, 0.81)**	**8858.956**	**2753**	**3352.956**
CE[2]	-	0.16 (0.10, 0.22)	0.84 (0.78, 0.90)	8871.542	2753	3365.542
E	-	-	1.00 (1.00, 1.00)	8900.797	2754	3392.797
Oranges						
Sat				8039.894	2848	2343.894
ACE[1]	0.39 (0.25, 0.46)	0.00 (0.00, 0.10)	0.61 (0.54, 0.67)	8043.710	2851	2341.710
AE[2]	**0.39 (0.33, 0.46)**	**-**	**0.61 (0.54, 0.67)**	**8043.710**	**2852**	**2339.710**
CE[2]	-	0.26 (0.21, 0.31)	0.74 (0.69, 0.79)	8062.866	2852	2358.866
E	-	-	1.00 (1.00, 1.00)	8148.966	2853	2442.966
Grapes						
Sat				6789.618	2846	1097.618
ACE[1]	0.36 (0.27, 0.43)	0.00 (0.00, 0.06)	0.64 (0.57, 0.71)	6809.582	2849	1111.582
AE[2]	**0.36 (0.29, 0.43)**	**-**	**0.64 (0.57, 0.71)**	**6809.582**	**2850**	**1109.582**
CE[2]	-	0.23 (0.18, 0.28)	0.77 (0.72, 0.82)	6834.820	2850	1134.820
E	-	-	1.00 (1.00, 1.00)	6901.461	2851	1199.461

Food item	Additive genetic effect (A)	Shared environment effect (C)	Nonshared environment effect (E)	-2LL[3]	Df[3]	AIC[3]
Carrots						
Sat				8086.426	2842	2402.426
ACE[1]	0.26 (0.15, 0.33)	0.00 (0.00, 0.08)	0.74 (0.67, 0.81)	8095.553	2845	2405.553
AE[2]	**0.26 (0.19, 0.33)**	**-**	**0.74 (0.67, 0.81)**	**8095.553**	**2846**	**2403.553**
CE[2]	-	0.17 (0.15, 0.22)	0.83 (0.78, 0.85)	8107.846	2846	2415.846
E	-	-	1.00 (1.00, 1.00)	8143.404	2847	2449.404
Green beans						
Sat				9076.185	2807	3462.185
ACE[1]	0.23 (0.14, 0.31)	0.00 (0.00, 0.05)	0.77 (0.69, 0.85)	9087.237	2810	3467.237
AE[2]	**0.23 (0.15, 0.31)**	**-**	**0.77 (0.69, 0.85)**	**9087.237**	**2811**	**3465.237**
CE[2]	-	0.13 (0.07, 0.18)	0.87 (0.82, 0.93)	9101.163	2811	3479.163
E	-	-	1.00 (1.00, 1.00)	9120.476	2812	3496.476
Cucumber						
Sat				9375.050	2834	3707.050
ACE[1]	0.33 (0.20, 0.40)	0.00 (0.00, 0.09)	0.67 (0.60, 0.74)	9379.711	2837	3705.711
AE[2]	**0.33 (0.26, 0.40)**	**-**	**0.67 (0.60, 0.74)**	**9379.711**	**2838**	**3703.711**
CE[2]	-	0.22 (0.16, 0.27)	0.78 (0.73, 0.84)	9394.588	2838	3718.588
E	-	-	1.00 (1.00, 1.00)	9452.156	2839	3774.156
Celery						
Sat				10003.67	2758	4487.669
ACE[1]	0.46 (0.26, 0.52)	0.00 (0.00, 0.15)	0.54 (0.48, 0.62)	10006.63	2761	4484.634
AE[2]	**0.46 (0.39, 0.52)**	**-**	**0.54 (0.48, 0.61)**	**10006.63**	**2762**	**4482.634**
CE[2]	-	0.32 (0.26, 0.37)	0.68 (0.63, 0.74)	10026.92	2762	4502.916
E	-	-	1.00 (1.00, 1.00)	10149.51	2763	4623.510
Mushrooms						
Sat				10770.80	2817	5136.802
ACE[1]	0.42 (0.33, 0.48)	0.00 (0.00, 0.06)	0.58 (0.52, 0.65)	10775.31	2820	5135.311
AE[2]	**0.42 (0.35, 0.48)**	**-**	**0.58 (0.52, 0.65)**	**10775.31**	**2821**	**5133.311**
CE[2]	-	0.26 (0.21, 0.32)	0.74 (0.68, 0.79)	10805.21	2821	5163.207
E	-	-	1.00 (1.00, 1.00)	10890.46	2822	5246.463
Parsnips						
Sat				10146.02	2765	4616.018
ACE[1]	0.43 (0.35, 0.50)	0.00 (0.00, 0.05)	0.57 (0.50, 0.64)	10154.94	2768	4618.938
AE[2]	**0.43 (0.36, 0.50)**	**-**	**0.57 (0.50, 0.64)**	**10154.94**	**2769**	**4616.938**
CE[2]	-	0.26 (0.20, 0.31)	0.74 (0.69, 0.80)	10191.28	2769	4653.278
E	-	-	1.00 (1.00, 1.00)	10270.36	2770	4730.358
Peas						
Sat				9345.844	2839	3667.844
ACE[1]	0.23 (0.14, 0.30)	0.00 (0.00, 0.07)	0.77 (0.70, 0.85)	9349.353	2842	3665.353
AE[2]	**0.23 (0.16, 0.30)**	**-**	**0.77 (0.70, 0.85)**	**9349.353**	**2843**	**3663.353**
CE[2]	-	0.14 (0.08, 0.19)	0.86 (0.81, 0.92)	9359.689	2843	3673.689
E	-	-	1.00 (1.00, 1.00)	9383.234	2844	3695.234
Sweetcorn						
Sat				8969.364	2833	3303.364
ACE[1]	0.29 (0.18, 0.36)	0.00 (0.00, 0.07)	0.71 (0.64, 0.79)	8977.853	2836	3305.853
AE[2]	**0.29 (0.21, 0.36)**	**-**	**0.71 (0.64, 0.79)**	**8977.853**	**2837**	**3303.853**
CE[2]	-	0.17 (0.11, 0.22)	0.83 (0.78, 0.89)	8992.295	2837	3318.295
E	-	-	1.00 (1.00, 1.00)	9026.559	2838	3350.559

Food item	Additive genetic effect (A)	Shared environment effect (C)	Nonshared environment effect (E)	-2LL[3]	Df[3]	AIC[3]
Broccoli						
Sat				9098.603	2822	3454.603
ACE[1]	0.33 (0.30, 0.40)	0.00 (0.00, 0.05)	0.67 (0.60, 0.70)	9106.205	2825	3456.205
AE[2]	**0.33 (0.29, 0.40)**	**-**	**0.67 (0.60, 0.71)**	**9106.205**	**2826**	**3454.205**
CE[2]	-	0.19 (0.14, 0.25)	0.81 (0.75, 0.86)	9128.262	2826	3476.262
E	-	-	1.00 (1.00, 1.00)	9173.445	2827	3519.445
Salad						
Sat				8348.106	2839	2670.106
ACE[1]	0.28 (0.22, 0.35)	0.00 (0.00, 0.05)	0.72 (0.65, 0.80)	8356.041	2842	2672.041
AE[2]	**0.28 (0.24, 0.35)**	**-**	**0.72 (0.65, 0.76)**	**8356.041**	**2843**	**2670.041**
CE[2]	-	0.17 (0.16, 0.22)	0.83 (0.78, 0.84)	8373.445	2843	2687.445
E	-	-	1.00 (1.00, 1.00)	8409.077	2844	2721.077
Red Pepper						
Sat				9298.561	2816	3666.561
ACE[1]	0.39 (0.31, 0.45)	0.00 (0.00, 0.05)	0.61 (0.55, 0.69)	9309.677	2819	3671.677
AE[2]	**0.39 (0.31, 0.45)**	**-**	**0.61 (0.55, 0.69)**	**9309.677**	**2820**	**3669.677**
CE[2]	-	0.22 (0.17, 0.28)	0.78 (0.72, 0.83)	9338.079	2820	3698.079
E	-	-	1.00 (1.00, 1.00)	9398.713	2821	3756.713
Raw tomato						
Sat				10804.55	2831	5142.546
ACE[1]	0.41 (0.34, 0.48)	0.00 (0.00, 0.04)	0.59 (0.52, 0.66)	10815.41	2834	5147.410
AE[2]	**0.41 (0.34, 0.48)**	**-**	**0.59 (0.52, 0.66)**	**10815.41**	**2835**	**5145.410**
CE[2]	-	0.24 (0.19, 0.29)	0.76 (0.71, 0.81)	10851.85	2835	5181.853
E	-	-	1.00 (1.00, 1.00)	10923.88	2836	5251.880
Beetroot						
Sat				9862.769	2672	4518.769
ACE[1]	0.52 (0.41, 0.58)	0.00 (0.00, 0.11)	0.48 (0.42, 0.54)	9863.324	2675	4513.324
AE[2]	**0.52 (0.46, 0.58)**	**-**	**0.48 (0.42, 0.54)**	**9863.324**	**2676**	**4511.324**
CE[2]	-	0.35 (0.30, 0.40)	0.65 (0.60, 0.70)	9895.997	2676	4543.997
E	-	-	1.00 (1.00, 1.00)	10036.726	2677	4682.726
Brussel Sprouts						
Sat				10378.68	2792	4794.681
ACE[1]	0.43 (0.36, 0.49)	0.00 (0.00, 0.06)	0.57 (0.51, 0.64)	10383.78	2793	4793.778
AE[2]	**0.43 (0.36, 0.49)**	**-**	**0.57 (0.51, 0.64)**	**10383.78**	**2794**	**4791.778**
CE[2]	-	0.27 (0.22, 0.32)	0.73 (0.68, 0.78)	10415.79	2794	4823.794
E	-	-	1.00 (1.00, 1.00)	10504.68	2795	4910.683
Vegetable Soup						
Sat				9424.132	2795	3834.132
ACE[1]	0.38 (0.29, 0.44)	0.00 (0.00, 0.05)	0.62 (0.56, 0.69)	9435.558	2798	3839.558
AE[2]	**0.38 (0.31, 0.44)**	**-**	**0.62 (0.56, 0.70)**	**9435.558**	**2799**	**3837.558**
CE[2]	-	0.24 (0.18, 0.29)	0.76 (0.71, 0.82)	9463.509	2799	3865.509
E	-	-	1.00 (1.00, 1.00)	9527.264	2800	3927.264
Coriander						
Sat				8792.655	2588	3616.655
ACE[1]	0.43 (0.33, 0.50)	0.00 (0.00, 0.07)	0.57 (0.51, 0.64)	8796.727	2591	3614.727
AE[2]	**0.43 (0.36, 0.50)**	**-**	**0.57 (0.50, 0.64)**	**8796.727**	**2592**	**3612.727**
CE[2]	-	0.27 (0.21, 0.33)	0.73 (0.67, 0.79)	8823.654	2592	3639.654
E	-	-	1.00 (1.00, 1.00)	8897.636	2593	3711.636

Food item	Additive genetic effect (A)	Shared environment effect (C)	Nonshared environment effect (E)	-2LL[3]	Df[3]	AIC[3]
Butter						
Sat				8365.026	2785	2795.026
ACE [1]	0.26 (0.15, 0.33)	0.00 (0.00, 0.07)	0.74 (0.67, 0.82)	8368.888	2788	2792.888
AE[2]	**0.26 (0.18, 0.33)**	**-**	**0.74 (0.67, 0.82)**	**8368.888**	**2789**	**2790.888**
CE[2]	-	0.16 (0.10, 0.21)	0.84 (0.79, 0.90)	8380.604	2789	2802.604
E[2]	-	-	1.00 (1.00, 1.00)	8408.574	2790	2828.574
Butter-like spread						
Sat				8647.343	2823	3001.343
ACE[1]	0.37 (0.27, 0.44)	0.00 (0.00, 0.06)	0.63 (0.56, 0.70)	8653.058	2826	3001.058
AE[2]	**0.37 (0.30, 0.44)**	**-**	**0.63 (0.56, 0.70)**	**8653.058**	**2827**	**2999.058**
CE[2]	-	0.23 (0.17, 0.28)	0.77 (0.72, 0.83)	8675.948	2827	3021.948
E	-	-	1.00 (1.00, 1.00)	8738.345	2828	3082.345
Cream						
Sat				9071.395	2782	3507.395
ACE[1]	0.20 (0.11, 0.28)	0.00 (0.00, 0.05)	0.80 (0.72, 0.89)	9083.610	2785	3513.610
AE[2]	**0.20 (0.11, 0.28)**	**-**	**0.80 (0.72, 0.89)**	**9083.610**	**2786**	**3511.610**
CE[2]	-	0.10 (0.04, 0.16)	0.90 (0.84, 0.96)	9093.284	2786	3521.284
E	-	-	1.00 (1.00, 1.00)	9103.907	2787	3529.907
Mayonnaise						
Sat				9853.247	2807	4239.247
ACE[1]	0.45 (0.38, 0.51)	0.00 (0.00, 0.06)	0.55 (0.49, 0.62)	9858.602	2810	4238.602
AE[2]	**0.45 (0.38, 0.51)**	**-**	**0.55 (0.49, 0.62)**	**9858.602**	**2811**	**4236.602**
CE[2]	-	0.28 (0.22, 0.33)	0.72 (0.67, 0.78)	9893.792	2811	4271.792
E	-	-	1.00 (1.00, 1.00)	9988.203	2812	4364.203
Plain biscuits						
Sat				7471.347	2845	1781.347
ACE[1]	0.34 (0.12, 0.43)	0.02 (0.00, 0.18)	0.64 (0.57, 0.73)	7490.648	2848	1794.648
AE[2]	**0.37 (0.29, 0.44)**	**-**	**0.63 (0.57, 0.71)**	**7490.722**	**2849**	**1792.722**
CE[2]	-	0.24 (0.19, 0.30)	0.76 (0.70, 0.81)	7499.357	2849	1801.357
E	-	-	1.00 (1.00, 1.00)	7567.613	2850	1867.613
Chocolate biscuits						
Sat				6411.041	2845	721.0410
ACE[1]	0.26 (0.11, 0.34)	0.00 (0.00, 0.10)	0.74 (0.66, 0.82)	6455.597	2848	759.5968
AE[2]	**0.26 (0.18, 0.34)**	**-**	**0.74 (0.66, 0.82)**	**6455.597**	**2849**	**757.5968**
CE[2]	-	0.16 (0.11, 0.22)	0.84 (0.78, 0.89)	6464.126	2849	766.1264
E	-	-	1.00 (1.00, 1.00)	6494.320	2850	794.3204
Cake						
Sat				6967.982	2845	1277.982
ACE[1]	0.07 (0.00, 0.26)	0.11 (0.00, 0.21)	0.82 (0.74, 0.90)	6968.759	2848	1272.759
AE[2]	**0.21 (0.13, 0.28)**	**-**	**0.79 (0.72, 0.87)**	**6970.264**	**2849**	**1272.264**
CE[2]	-	0.16 (0.10, 0.21)	0.85 (0.79, 0.90)	6969.146	2849	1271.146
E	-	-	1.00 (1.00, 1.00)	6997.604	2850	1297.604
Apple pie						
Sat				9931.490	2818	4295.490
ACE[1]	0.45 (0.38, 0.51)	0.00 (0.00, 0.08)	0.55 (0.49, 0.62)	9932.469	2821	4290.469
AE[2]	**0.45 (0.38, 0.51)**	**-**	**0.55 (0.49, 0.62)**	**9932.469**	**2822**	**4288.469**
CE[2]	-	0.30 (0.24, 0.35)	0.70 (0.65, 0.76)	9957.550	2822	4313.550
E	-	-	1.00 (1.00, 1.00)	10065.838	2823	4419.838

Food item	Additive genetic effect (A)	Shared environment effect (C)	Nonshared environment effect (E)	-2LL[3]	Df[3]	AIC[3]
Ice Cream						
Sat				6831.304	2842	1147.304
ACE [1]	0.27 (0.17, 0.34)	0.00 (0.00, 0.07)	0.73 (0.66, 0.80)	6837.031	2845	1147.031
AE[2]	**0.27 (0.20, 0.34)**	**-**	**0.73 (0.66, 0.80)**	**6837.031**	**2846**	**1145.031**
CE[2]	-	0.17 (0.11, 0.22)	0.83 (0.78, 0.89)	6848.051	2846	1156.051
E[2]	-	-	1.00 (1.00, 1.00)	6882.356	2847	1188.356
Custard						
Sat				9447.313	2784	3879.313
ACE[1]	0.47 (0.35, 0.53)	0.00 (0.00, 0.09)	0.53 (0.47, 0.59)	9448.633	2787	3874.633
AE[2]	**0.47 (0.41, 0.53)**	**-**	**0.53 (0.47, 0.59)**	**9448.633**	**2788**	**3872.633**
CE[2]	-	0.31 (0.26, 0.36)	0.69 (0.64, 0.74)	9479.303	2788	3903.303
E	-	-	1.00 (1.00, 1.00)	9600.222	2789	4022.222
Chocolate						
Sat	Sat			16291.41	2846	10599.41
ACE[1]	0.25 (0.14, 0.33)	0.00 (0.00, 0.07)	0.75 (0.67, 0.83)	5446.417	2849	-251.5831
AE[2]	**0.25 (0.17, 0.33)**	**-**	**0.75 (0.67, 0.83)**	**5446.417**	**2850**	**-253.5831**
CE[2]	-	0.15 (0.09, 0.21)	0.85 (0.79, 0.91)	5457.526	2850	-242.4739
E	-	-	1.00 (1.00, 1.00)	5482.869	2851	-219.1308
Crisps						
Sat				6972.759	2846	1280.759
ACE[1]	0.34 (0.26, 0.41)	0.00 (0.00, 0.09)	0.66 (0.59, 0.74)	6982.397	2849	1284.397
AE[2]	**0.34 (0.26, 0.41)**	**-**	**0.66 (0.59, 0.74)**	**6982.397**	**2850**	**1282.397**
CE[2]	-	0.20 (0.15, 0.26)	0.80 (0.74, 0.85)	7001.619	2850	1301.619
E	-	-	1.00 (1.00, 1.00)	7050.525	2851	1348.525
Peanut butter						
Sat				10054.72	2654	4746.721
ACE[1]	0.49 (0.30, 0.56)	0.01 (0.00, 0.16)	0.50 (0.44, 0.57)	10055.26	2657	4741.261
AE[2]	**0.50 (0.43, 0.56)**	**-**	**0.50 (0.44, 0.57)**	**10055.26**	**2658**	**4739.265**
CE[2]	-	0.34 (0.29, 0.39)	0.66 (0.61, 0.71)	10078.40	2658	4762.396
E	-	-	1.00 (1.00, 1.00)	10211.40	2659	4893.403
Gummy sweets						
Sat				8533.489	2824	2885.489
ACE[1]	0.40 (0.31, 0.47)	0.00 (0.00, 0.06)	0.60 (0.53, 0.67)	8540.938	2827	2886.938
AE[2]	**0.40 (0.33, 0.47)**	**-**	**0.60 (0.53, 0.67)**	**8540.938**	**2828**	**2884.938**
CE[2]	-	0.25 (0.19, 0.30)	0.75 (0.70, 0.81)	8566.693	2828	2910.693
E	-	-	1.00 (1.00, 1.00)	8640.388	2829	2982.388

Standard Maximum Likelihood Structural Equation Modelling (MLSEM) was used to derive estimates of A, C and E, as well as provide two goodness-of-fit statistics; -2LL and the AIC respectively. The selection of the most parsimonious model was indicated by the lowest absolute value of the AIC and smallest $\Delta\chi 2$.

[1] The full ACE model was nested within the saturated model
[2] Sub-models were nested within the full ACE model
[3] Abbreviations; *- 2LL= -2 times log-likelihood of data, df=degrees of freedom, AIC=Akaike Information Criterion (AIC)*

Appendix D3 Model fit and parameter estimates for the saturated, ADE model and sub models of food category preferences

Food category	Additive genetic effect (A)	Dominant genetic effect (D)	Nonshared environment effect (E)	-2LL[3]	Df[3]	AIC[3]	Δ -2LL
Vegetables[4]							
Sat				6109.386	2856	397.3862	
ADE[1]	0.54 (0.42, 0.62)	0.03 (0.00, 0.12)	0.46 (0.41, 0.52)	6110.895	2859	392.8953	1.509
AE[2]	**0.58 (0.52, 0.63)**	**-**	**0.42 (0.37, 0.48)**	**6111.381**	**2860**	**391.3814**	**0.486**
DE[2]	-	0.32 (0.27, 0.37)	0.68 (0.63, 0.73)	6183.445	2860	463.4448	72.549
E[2]	-	-	1.00 (1.00, 1.00)	6316.762	2861	594.7621	205.867
Fruit[5]							
Sat				6310.121	2854	602.1213	
ADE[1]	**0. 35 (0.29, 0.46)**	**0. 15 (0.06, 0.24)**	**0.50 (0.44, 0.57)**	**6315.440**	**2857**	**601.3774**	**5.256**
AE[2]	0.51 (0.45, 0.57)	-	0.51 (0.45, 0.57)	6324.980	2858	608.9802	9.603
DE[2]	-	0.35 (0.30, 0.40)	0.65 (0.60, 0.70)	6344.889	2858	628.8892	29.512
E[2]	-	-	1.00 (1.00, 1.00)	6500.514	2859	782.5141	185.137
Meat/Fish[6]							
Sat				5864.102	2846	172.1023	
ADE[1]	0. 39 (0.27, 0.52)	0.08 (0.00, 0.17)	0.53 (0.46, 0.60)	5868.383	2849	172.3828	4.281
AE[2]	**0.48 (0.41, 0.54)**	**-**	**0.52 (0.46, 0.59)**	**5870.868**	**2850**	**170.8679**	**2.485**
DE[2]	-	0.28 (0.22, 0.33)	0.72 (0.67, 0.78)	5899.942	2850	199.9424	31.56
E[2]	-	-	1.00 (1.00, 1.00)	5992.453	2851	290.4527	124.07
Dairy[7]							
Sat				6059.620	2855	349.6199	
ADE[1]	0.42 (0.29, 0.53)	0.05 (0.00, 0.15)	0.53 (0.47, 0.60)	6061.036	2858	345.0356	1.416
AE[2]	**0.48 (0.41, 0.54)**	**-**	**0.52 (0.46, 0.59)**	**6062.200**	**2859**	**344.2002**	**1.165**
DE[2]	-	0.28 (0.23, 0.33)	0.72 (0.67, 0.77)	6099.114	2859	381.1137	38.078
E[2]	-	-	1.00 (1.00, 1.00)	6198.533	2860	478.5330	137.497
Snacks							
Sat				4366.211	2856	-1345.79	
ADE[1]	0.41 (0.28, 0.52)	0.05 (0.00, 0.15)	0.54 (0.48, 0.61)	4375.872	2859	-1342.13	9.661
AE[2]	**0.47 (0.40, 0.53)**	**-**	**0.53 (0.47, 0.60)**	**4376.928**	**2860**	**-1343.07**	**1.055**
DE[2]	-	0.27 (0.22, 0.32)	0.73 (0.68, 0.78)	4410.221	2860	-1309.78	34.348
E[2]	-	-	1.00 (1.00, 1.00)	4498.200	2861	-1223.80	122.3272
Starches[8]							
Sat				5848.940	2856	136.9400	
ADE[1]	0.37 (0.25 0.46)	0.00 (0.00, 0.08)	0.63 (0.61, 0.71)	5854.182	2859	136.1816	5.242
AE[2]	**0.37 (0.29, 0.45)**	**-**	**0.63 (0.55, 0.71)**	**5854.182**	**2860**	**134.1816**	**0**
DE[2]	-	0.18 (0.13, 0.24)	0.82 (0.76, 0.87)	5881.719	2860	161.7192	27.537
E[2]	-	-	1.00 (1.00, 1.00)	5922.439	2861	200.4394	68.257

Standard Maximum Likelihood Structural Equation Modelling (MLSEM) was used to derive estimates of A, C and E, as well as provide two goodness-of-fit statistics; -2LL and the AIC respectively. The selection of the most parsimonious model was indicated by the lowest absolute value of the AIC and smallest $\Delta\chi 2$.

[1] The full ADE model was nested within the saturated model
[2] Sub-models were nested within the full ADE model
[3] *Abbreviations; - 2LL= -2 times log-likelihood of data, Δ -2LL= Change in the -2 times log likelihood of data, df= degrees of freedom, AIC= Akaike Information Criterion*
[4] excludes observations for celery liking from individuals self-reporting an allergy against celery.
[5] excludes observations for strawberries, apples and oranges for individuals self-reporting a strawberry, apple or orange allergy.
[6] excludes observations for all meat items from self-reported pescetarians, vegetarians and vegan. White fish, oily fish, tinned tuna and smoked salmon liking includes pescetarians' observations but excludes preference scores from individuals reporting a fish allergy
[7] excludes observation for egg liking from individuals reporting an egg allergy and vegans. Food preference scores for soft cheese, hard cheese, butter, cream, yoghurt, cottage cheese and custard were excluded from vegans and individuals self-reporting a dairy allergy.
[8] excludes observations for wheat cereal from individuals reporting a wheat/gluten allergy

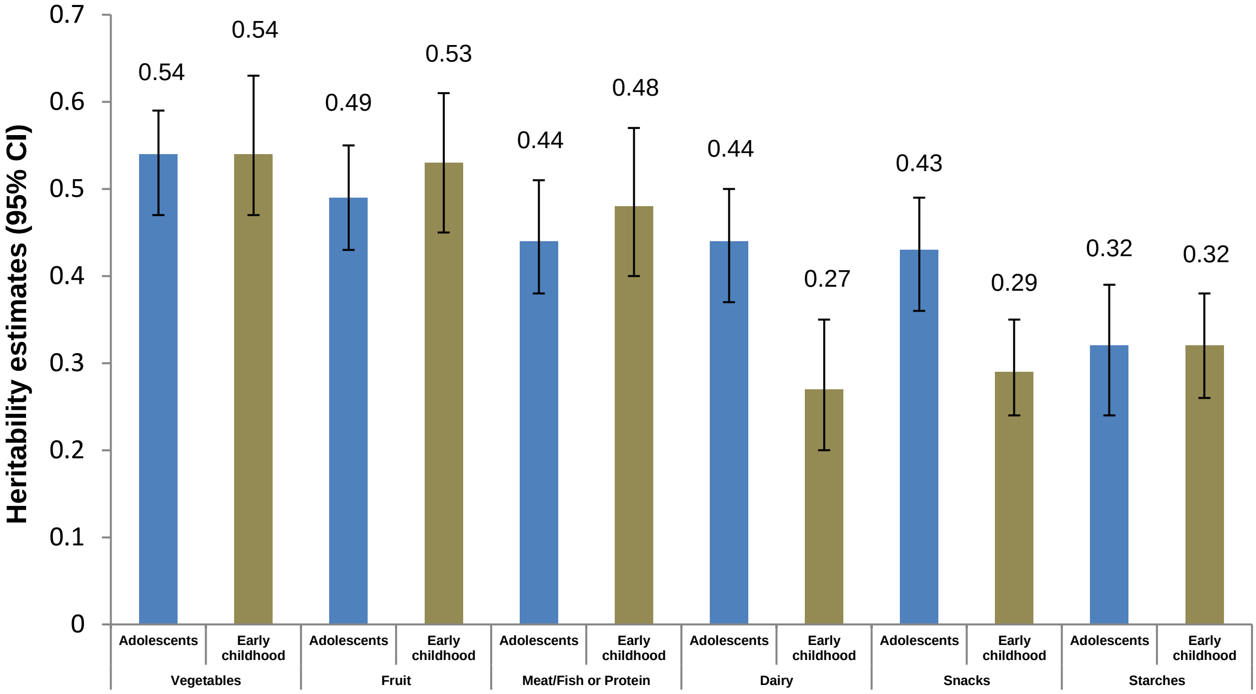
Appendix D4 Model fit and parameter estimates for the saturated, ACE model and submodels of food preferences excluding all observations from TEDS participants categorized as pescetarians, vegetarians, vegans or self-reported food allergies

Food category	Additive genetic effect (A)	Shared environment effect (C)	Nonshared environment effect (E)	-2LL[3]	Df[3]	BIC[3]
Vegetables						
Sat				6329.102	2299	-5027.810
ACE [1]	0.54 (0.45, 0.60)	0.00 (0.00, 0.06)	0.45 (0.13, 0.17)	6334.023	2304	-5043.167
CE[2]	-	0.35 (0.29, 0.40)	0.65 (0.60, 0.71)	6382.774	2305	-4604.736
AE[2]	**0.55 (0.48, 0.60)**	**-**	**0.45 (0.40, 0.52)**	**6334.023**	**2305**	**-5046.730**
E[2]	-	-	1.00 (1.00, 1.00)	6518.468	2306	-4958.071
Fruit						
Sat				6425.249	2298	-4976.173
ACE [1]	0.41 (0.21, 0.54)	0.06 (0.00, 0.21)	0.53 (0.46, 0.60)	6431.420	2303	-4460.175
CE[2]	-	0.36 (0.30, 0.41)	0.64 (0.59 ,0.70)	6447.581	2304	-4366.514
AE[2]	**0.48 (0.42, 0.54)**	**-**	**0.52 (0.46, 0.58)**	**6431.939**	**2304**	**-4994.209**
E[2]	-	-	1.00 (1.00, 1.00)	6588.245	2305	-4919.619
Meat/Fish						
Sat				6373.927	2299	-5005.397
ACE[1]	0.48 (0.35, 0.55)	0.00 (0.00, 0.10)	0.52 (0.45, 0.59)	6376.772	2304	-5021.792
CE[2]	-	0.32 (0.41, 0.50)	0.54 (0.50, 0.59)	6404.454	2305	-5011.515
AE[2]	**0.49 (0.42, 0.55)**	**-**	**0.52 (0.45, 0.59)**	**6376.772**	**2305**	**-5025.356**
E[2]	-	-	1.00 (1.00, 1.00)	6512.751	2306	-4960.929
Dairy						
Sat				6393.926	2299	-4995.398
ACE [1]	0.45 (0.33, 0.51)	0.00 (0.00, 0.08)	0.55 (0.49, 0.62)	6398.550	2304	-5010.903
CE[2]	-	0.29 (0.24, 0.35)	0.71 (0.65, 0.76)	6425.587	2305	-5000.948
AE[2]	**0.45 (0.38, 0.51)**	**-**	**0.55 (0.49, 0.62)**	**6398.550**	**2305**	**-5014.466**
E[2]	-	-	1.00 (1.00, 1.00)	6519.198	2306	-4957.706
Snacks						
Sat				6467.658	2299	-4958.532
ACE [1]	0.41(0.27, 0.48)	0.00 (0.00, 0.10)	0.59 (0.52, 0.67)	6489.264	2304	-4965.546
CE[2]	-	0.26 (0.21, 0.32)	0.74 (0.68, 0.79)	6508.619	2305	-4959.432
AE[2]	**0.41 (0.33, 0.48)**	**-**	**0.59 (0.52, 0.67)**	**6489.264**	**2305**	**-4969.110**
E[2]	-	-	1.00 (1.00, 1.00)	6581.813	2306	-4926.398
Starch						
Sat				6505.276	2299	-4939.723
ACE [1]	0.32 (0.21, 0.40)	0.00 (0.00, 0.06)	0.68 (0.60, 0.77)	6515.695	2304	-4952.330
CE[2]	-	0.18 (0.12, 0.24)	0.82 (0.76, 0.88)	6532.634	2305	-4947.425
AE[2]	**0.32 (0.23, 0.40)**	**-**	**0.68 (0.60, 0.77)**	**6515.695**	**2305**	**-4955.894**
E[2]	-	-	1.00 (1.00, 1.00)	6567.452	2306	-4933.579

[1] The full ACE model was nested within the saturated model
[2] Sub-models were nested within the full ACE model
[3] Abbreviations; - 2LL= -2 times log-likelihood of data, df=degrees of freedom, AIC=Akaike Information Criterion
[4] Cronbach α calculated for the sample excl. all self-reported pescetarians, vegetarians, vegans and individuals self-reporting a food allergy (n=2507).

Appendix D5 Comparison of genetic influences on food preference categories in an adolescent[1] and paediatric[2] sample

[1] Estimates of the percentage in food preference variation explained by genetic factors in this graph are based on



2865 participants of the adolescent TEDS twin cohort. Food preference data were ascertained by self-report using a food preference questionnaire when the participants were 19 years old.
[2] Estimates of the percentage in food preference variation explained by genetic factors in this graph are based on 2686 participants of the paediatric Gemini twin cohort. Food preference data were collected from a parent-completed food preference questionnaire when the participants were 3 years old. Data for this part of the figure is based on a previous publication by Fildes et al. (2014).

Appendix D6 Model fit and parameter estimates for the saturated, ADE model and submodels of beverage preferences

Beverage type	Additive genetic effect (A)	Dominant genetic effect (D)	Nonshared environment effect (E)	-2LL[3]	Df[3]	AIC[3]	Δ -2LL	p-value
SSBs[3]								
Sat				9609.825	2832	3945.825		
ADE[1]	0.30 (0.24, 0.43)	0.08 (0.00, 0.17)	0.62 (0.55, 0.70)	9614.131	2835	3944.131	4.305359	0.23
AE[2]	**0.39 (0.32, 0.46)**	**-**	**0.61 (0.54, 0.68)**	**9616.851**	**2836**	**3944.851**	**2.719993**	**0.10**
DE[2]	-	0.24 (0.19, 0.29)	0.76 (0.71, 0.81)	9631.547	2836	3959.547	17.416468	<0.001
E[2]	-	-	1.00 (1.00, 1.00)	9703.572	2837	4029.572	89.441158	<0.001
NNSBs[3]								
Sat				9545.841	2818	3909.841		
ADE[1]	**0.23 (0.10, 0.36)**	**0.16 (0.07, 0.25)**	**0.61 (0.53, 0.68)**	**9546.322**	**2821**	**3904.322**	**4.804889**	**0.92**
AE[2]	0.43 (0.36, 0.49)	-	0.57 (0.51, 0.64)	9558.221	2822	3914.221	11.899122	<0.001
DE[2]	-	0.28 (0.23, 0.33)	0.72 (0.67, 0.77)	9557.576	2822	3913.576	11.253711	<0.001
E[2]	-	-	1.00 (1.00, 1.00)	9660.699	2823	4014.699	114.37726	<0.001
Orange juice								
Sat				7844.431	2840	2164.431		
ADE[1]	0.24 (0.18, 0.32)	0.00 (0.00, 0.04)	0.76 (0.68, 0.83)	7854.672	2843	2168.672	10.241	0.02
AE[2]	**0.24 (0.20, 0.32)**	**-**	**0.76 (0.68, 0.80)**	**7854.672**	**2844**	**2166.672**	**0**	**1.00**
DE[2]	-	0.08 (0.03, 0.14)	0.92 (0.86, 0.97)	7873.266	2844	2185.266	18.594	<0.001
E[2]	-	-	1.00 (1.00, 1.00)	7881.523	2845	2191.523	26.851	<0.001
Fruit squash								
Sat				8031.215	2838	2355.215		
ADE[1]	**0.28 (0.15, 0.41)**	**0.14 (0.05, 0.23)**	**0.58 (0.51, 0.65)**	**8034.727**	**2841**	**2352.727**	**3.511726**	**0.32**
AE[2]	0.44 (0.38, 0.51)	-	0.56 (0.49, 0.62)	8043.012	2842	2359.012	8.285402	<0.001
DE[2]	-	0.29 (0.24, 0.34)	0.71 (0.66, 0.76)	8051.672	2842	2367.672	16.944929	<0.001
E[2]	-	-	1.00 (1.00, 1.00)	8157.514	2843	2471.514	122.78661	<0.001
Milk								
Sat				7269.105	2698	1873.105		
ADE[1]	0.36 (0.21, 0.44)	0.00 (0.00, 0.11)	0.64 (0.56, 0.72)	7277.283	2701	1875.283	8.1783824	0.04
AE[2]	**0.36 (0.28, 0.44)**	**-**	**0.64 (0.56, 0.72**	**7277.284**	**2702**	**1873.284**	**0.0011715**	**0.97**
DE[2]	-	0.20 (0.14, 0.26)	0.80 (0.74, 0.86)	7298.526	2702	1894.526	21.243401	<0.001
E[2]	-	-	1.00 (1.00, 1.00)	7340.874	2703	1934.874	63.591339	<0.001
Tea								
Sat				7109.816	2409	2291.816		
ADE[1]	0.51 (0.42, 0.58)	0.00 (0.00, 0.03)	0.49 (0.42, 0.58)	7109.816	2410	2289.816	11.63	1.00
AE[2]	**0.50 (0.42, 0.58)**	**-**	**0.50 (0.42, 0.58)**	**7171.829**	**2410**	**2351.829**	**0**	**<0.001**
DE[2]	-	0.19 (0.12, 0.26)	0.81 (0.74, 0.88)	7201.412	2411	2379.412	62.01	<0.001
E[2]	-	-	1.00 (1.00, 1.00)	7201.412	2411	2379.412	91.6	<0.001
Coffee								
Sat				6351.94	1896	2559.94		
ADE[1]	0.33 (0.14, 0.44)	0.00 (0.00, 0.12)	0.67 (0.56, 0.79)	6356.35	1899	2558.35	4.41	0.22
AE[2]	**0.33 (0.21, 0.44)**	**-**	**0.67 (0.56, 0.79)**	**6356.35**	**1900**	**2556.35**	**0.00**	**1.00**
DE[2]	-	0.17 (0.09, 0.25)	0.83 (0.75, 0.91)	6366.625	1900	2566.625	10.27	<0.001
E[2]	-	-	1.00 (1.00, 1.00)	6382.128	1901	2580.128	25.78	<0.001

Maximum Likelihood Structural Equation Modelling (MLSEM) was used to derive estimates of A, D and E, as well as provide two goodness-of-fit statistics; -2LL and the AIC respectively. The selection of the most parsimonious model was indicated by the lowest absolute value of the AIC and smallest $\Delta\chi 2$.

[1] The full ADE model was nested within the saturated model

[2] Sub-models were nested within the full ADE model

[3] *Abbreviations; - 2LL: -2 times log-likelihood of data, AIC: Akaike Information Criterion (AIC), df: degrees of freedom, NNSBs: Non-nutritive sweetened beverages, SSB: Sugar-sweetened beverages*

Appendix D7 Beverage preference scores and tetrachoric correlations (TTC) by zygosity

Beverage item	n[1] (%)[2]	Mean preference score[3] (SD)	Median preference score[3] (SD)	MZ[4] TCC[4] (95% CI)	DZ[4] TCC[4] (95% CI)
SSBs[4]	2841 (99.2)	3.73 (1.37)	4.00 (1.38)	0.47 (0.34, 0.59)	0.11 (0.00, 0.23)
NNSBs[4]	2827 (98.7)	3.64 (1.34)	4.00 (1.33)	0.48 (0.34, 0.59)	0.22 (0.11, 0.34)
Orange juice	2849 (99.4)	4.43 (0.97)	5.00 (0.97)	0.23 (0.05 0.39)	0.00 (0.00, 0.09)
Fruit cordial	2847 (99.3)	4.23 (1.02)	5.00 (1.02)	0.54 (0.42, 0.64)	0.20 (0.09, 0.31)
Milk	2707 (94.5)	4.22 (0.95)	5.00 (0.96)	0.60 (0.48, 0.70)	0.09 (0.02, 0.21)
Tea	2415 (84.3)	4.31 (1.08)	5.00 (1.08)	0.73 (0.62, 0.82)	0.04 (-0.09, 0.18)
Coffee	1905 (66.5)	3.85 (1.29)	4.00 (1.30)	0.48 (0.30, 0.60)	0.08 (-0.06, 0.25)

[1] Number of observations included in mean and median beverage liking score (excl. observations from individuals that never consume the specific beverage)
[2] Percentage of the full sample that reported occasional consumption of the beverage
[3] Preference scores were rated on a 5-point Likert scale, with a higher score indicating a higher preference for the beverage item.
[4] *Abbreviations: TCC=Tetrachoric Correlations; MZ= Monozygotic; DZ=Dizygotic; NNSBs=Non-nutritive sweetened beverages, SSB=Sugar-sweetened beverages*

Appendix D8 Threshold Model fit and parameter estimates for the saturated, ACE model and submodels of beverage preferences

Beverage type	Additive genetic effect (A)	Shared environment effect (C)	Nonshared environment effect (E)	-2LL[3]	Df[3]	AIC[3]	Δ -2LL	p-value
SSBs[1,3]								
Sat				3724.058	2830	-1935.94		
ACE[3]	0.42 (0.25, 0.52)	0.00 (0.00, 0.11)	0.58 (0.48, 1.00)	3737.736	2837	-1936.26	13.68	0.06
AE[4]	**0.42 (0.30, 0.52)**	**-**	**0.58 (0.48, 1.00)**	**3737.736**	**2838**	**-1938.26**	**0.00**	**1.00**
CE[4]	-	0.26 (0.17, 0.34)	0.74 (0.65, 1.00)	3750.700	2838	-1925.3	12.96	<0.001
E[4]	-	-	1.00 (1.00, 1.00)	3782.678	2839	-1895.32	44.94	<0.001
NNSBs[1,3]								
Sat				3510.043	2816	-2121.957		
ACE[3]	0.47 (0.15, 0.58)	0.00 (0.00, 0.24)	0.53 (0.42, 1.00)	3515.951	2823	-2130.05	5.91	0.55
AE[4]	**0.47 (0.41, 0.58)**	**-**	**0.53 (0.42, 1.00)**	**3515.951**	**2824**	**-2132.05**	**0.00**	**1.00**
CE[4]	-	0.33 (0.24, 0.41)	0.67 (0.59, 1.00)	3523.844	2824	-2124.16	7.89	<0.001
E[4]	-	-	1.00 (1.00, 1.00)	3572.634	2825	-2077.37	56.68	<0.001
Orange juice[2]								
Sat				2751.467	2495	-2238.53		
ACE[3]	0.23 (0.05, 0.39)	0.00 (0.00, 0.09)	0.77 (0.61, 0.95)	2902.347	2502	-2101.65	150.88	0.00
AE[4]	**0.23 (0.05, 0.39)**	**-**	**0.77 (0.61, 0.95)**	**2902.347**	**2503**	**-2103.65**	**0.00**	**1.00**
CE[4]	-	0.07 (0.00, 0.19)	0.92 (0.81, 1.00)	2907.614	2503	-2098.39	5.27	0.02
E[4]	-	-	1.00 (1.00, 1.00)	2908.933	2504	-2099.07	6.59	0.04
Fruit cordial[2]								
Sat				3868.794	2836	-1803.21		
ACE[3]	0.51 (0.40, 0.61)	0.00 (0.00, 0.14)	0.49 (0.39, 0.60)	3874.622	2843	-1811.38	5.83	0.56
AE[4]	**0.51 (0.40, 0.61)**	**-**	**0.49 (0.39, 0.60)**	**3874.622**	**2844**	**-1813.38**	**0.00**	**1.00**
CE[4]	-	0.33 (0.25, 0.41)	0.67 (0.59, 1.00)	3889.705	2844	-1798.30	15.08	<0.001
E[4]	-	-	1.00 (1.00, 1.00)	3946.426	2845	-1743.57	71.80	<0.001
Milk[2]								
Sat				3671.885	2696	-1720.12		
ACE[3]	0.51 (0.39, 0.61)	0.00 (0.00, 0.07)	0.49 (0.39, 1.00)	3685.956	2703	-1720.04	14.07	0.05
AE[4]	**0.51 (0.39, 0.61)**	**-**	**0.49 (0.39, 1.00)**	**3685.956**	**2704**	**-1722.04**	**0.00**	**1.00**
CE[4]	-	0.29 (0.20, 0.38)	0.71 (0.62, 1.00)	3709.498	2704	-1698.50	23.54	<0.001
E[4]	-	-	1.00 (1.00, 1.00)	3749.390	2705	-1660.61	63.43	<0.001
Tea[2]								
Sat				3142.318	2404	-1665.68		
ACE[3]	0.62 (0.50, 1.00)	0.00 (0.00, 0.06)	0.38 (0.27, 1.00)	3164.124	2411	-1657.88	21.81	0.00
AE[4]	**0.62 (0.50, 1.00)**	**-**	**0.38 (0.27, 1.00)**	**3164.124**	**2412**	**-1659.88**	**0.00**	**1.00**
CE[4]	-	0.34 (0.24, 0.43)	0.66 (0.57, 1.00)	3198.295	2412	-1625.71	34.17	<0.001
E[4]	-	-	1.00 (1.00, 1.00)	3240.230	2413	-1585.77	76.11	<0.001
Coffee[1]								
Sat				2541.410	1894	-1246.59		
ACE[3]	0.41 (0.25, 0.56)	0.00 (0.00, 0.01)	0.59 (0.00, 1.00)	2548.027	1901	-1253.97	6.62	0.47
AE[4]	**0.41 (0.25, 0.56)**	**-**	**0.59 (0.00, 1.00)**	**2548.027**	**1902**	**-1255.97**	**0.00**	**1.00**
CE[4]	-	0.25 (0.12, 0.36)	0.75 (0.64, 1.00)	2555.393	1902	-1248.61	7.37	0.01
E[4]	-	-	1.00 (1.00, 1.00)	2570.613	1903	-1235.39	22.59	<0.001

Maximum Likelihood Structural Equation Modelling (MLSEM) was used to derive estimates of A, C and E, as well as provide two goodness-of-fit statistics; -2LL and the AIC respectively. The selection of the most parsimonious model was indicated by the p=value and the lowest absolute value of the AIC.
[1] Preference score were split by the median value as =<4 vs >4
[2] Preference score were split by the median value as =<5 vs >=5
[3] The full ACE model was nested within the saturated model
[4] Sub-models were nested within the full ACE model
[5] *Abbreviations; - 2LL= -2 times log-likelihood of data, df=degrees of freedom, AIC=Akaike Information Criterion (AIC), NNSBs=Non-nutritive sweetened beverages, SSB=Sugar-sweetened beverages*

Appendix D9 Drink preference score intraclass correlations (ICC) by zygosity and sex

Drink item (n[1])	MZ[2] ICC[2] (95% CI) MM		MZ[2] ICC[2] (95% CI) FF		DZ ICC[2] (95% CI) MM		DZ[2] ICC[2] (95% CI) FF		DZ[2] ICC[2] (95% CI) os	
SSBs (n=2841)	0.29	(0.15, 0.43)	0.41	(0.31, 0.50)	0.27	(0.10, 0.41)	0.21	(0.09, 0.31)	0.00	(-0.11, 0.10)
NNSBs (n=2827)	0.36	(0.22, 0.49)	0.41	(0.31, 0.50)	0.21	(0.05, 0.37)	0.31	(0.20, 0.40)	0.16	(0.06, 0.26)
Fruit squash (n=2847)	0.36	(0.22, 0.49)	0.44	(0.35, 0.53)	0.23	(0.06, 0.39)	0.16	(0.06, 0.26)	0.27	(0.16, 0.37)
Orange juice (n=2849)	0.11	(-0.02, 0.26)	0.32	(0.21, 0.42)	-0.02	(-0.19, 0.15)	0.01	(-0.11, 0.11)	-0.04	(-0.15, 0.07)
Milk (n=2707)	0.26	(0.13, 0.40)	0.43	(0.32, 0.53)	-0.04	(-0.23, 0.15)	0.13	(0.01, 0.24)	0.04	(-0.09, 0.17)
Tea (n=2415)	0.11	(-0.04, 0.26)	0.32	(0.21, 0.42)	-0.02	(-0.19, 0.15)	0.01	(-0.11, 0.11)	-0.04	(-0.15, 0.07)
Coffee (n=1905)	0.26	(0.11, 0.40)	0.43	(0.32, 0.53)	-0.04	(-0.23, 0.15)	0.13	(0.01, 0.24)	0.04	(-0.09, 0.17)

Preference scores were rated on a 5-point Likert scale, with a higher score indicating a higher preference for the drink item.

[1] Number of observations included in mean drink liking score (excl. observations from individuals that never consuming the specific drink)

[2] *Abbreviations: ICCs=Intraclass Correlations; MZ=Monozygotic; DZ=Dizygotic; FF=same sex female pairs only; MM=same sex male pairs only; os=opposite-sex pairs only*

Appendix D10 Parameters estimates (95% Confidence intervals) for A, C and E for males and females considering qualitative and quantitative sex differences for the liking for sugar-sweetened beverages

Model	Male			Female				
	A_m[1]	C_m[1]	E_m[1]	A_f[1]	C_f[1]	E_f[1]	r_A[1]	r_C[1]
Full sex limitation (r_A=free)	0.11 (0.00-0.48)	0.26 (0.04-0.44)	0.63 (0.51-0.76)	0.36 (0.12-0.48)	0.04 (0.00-0.24)	0.60 (0.50-0.68)	0.24 (0.00-0.50)	1.00
Full sex limitation (r_C=free)	0.09 (0.00-0.37)	0.28 (0.06-0.44)	0.63 (0.51-0.76)	0.35 (0.08-0.48)	0.05 (0.00-0.27)	0.60 (0.52-0.69)	0.5	1.00 (0.33-1.00)
Common effects model (rA=0.5, rc=1)	0.09 (0.00-0.37)	0.28 (0.06-0.45)	0.63 (0.51-0.76)	0.35 (0.12-0.48)	0.05 (0.00-0.24)	0.60 (0.52-0.69)	0.5	1.00
	A		**C**		**E**		**Scalar**	
Scalar Model	0.35 (0.27-0.42)		0.00 (0.00-0.06)		0.65 (0.58-0.73)		1.09 (1.03-1.15)	
	A		**C**		**E**		**r_A**	**r_C**
Homogeneity model (no sex differences)	0.35 (0.27-0.42)		0.00 (0.00-0.06)		0.65 (0.58-0.73)		0.5	1.00

[1] Abbreviations: A= additive genetic component of variance; C=shared environmental component of variance; E=unique environmental component of variance; rA= genetic correlation, rC=shared environmental correlation, rE=non-shared environmental correlation.

Appendix D10.1 Heterogeneity model fit statistics for the liking of sugar-sweetened beverages

Model [1]	Comp.	Ep[1]	-2LL[1]	df[1]	AIC[1]	Δ-2LL	Δdf	p-value	ΔAIC
1 Saturated model		23	9626.144	2818	3990.144				
2 Full sex limitation	1	9	9699.952	2832	4035.952	73.808	14	<0.001	-45.808
3 Full sex limitation	1	9	9699.952	2832	4035.952	73.808	14	<0.001	-45.808
4 Common effects model	**2&3**	**8**	**9699.952**	**2833**	**4033.952**	**0.000**	**1**	**1.00**	**-2.000**
5 Scalar Model	4	6	9722.293	2835	4052.293	22.341	2	<0.001	-18.341
6 Homogeneity model	5	5	9732.027	2836	4060.027	9.734	1	0.002	-7.734

[1] Model 2 & 3 = Full sex-limitation model allowing quantitative and qualitative differences between males and females and constraining either rC or rA
[2] Model 4 = Common effects model where sex-specific pathways are constrained to zero (rA=0.5, rC=1)
[3] Model 5 = Scalar model where sex-specific effects removed, but the variance components for females are all constrained to be equal to a scalar multiple of the male variance components
[4] Model 6 = Homogeneity model which assumes no sex differences

Appendix D11 Parameters estimates (95% Confidence intervals) for A, C and E for males and females considering qualitative and quantitative sex differences in the liking for non-nutritive sweetened beverages

Model	Male			Female				
	A_m[1]	C_m[1]	E_m[1]	A_f[1]	C_f[1]	E_f[1]	r_A[1]	r_C[1]
Full sex limitation (r_A=free)	0.34 (0.05-0.50)	0.04 (0.00-0.32)	0.61 (0.50-0.26)	0.18 (0.08-0.46)	0.22 (0.08-0.41)	0.60 (0.51-0.70)	0.5 (0.39-0.50)	1.00
Full sex limitation (r_C=free)	0.34 (0.12-0.50)	0.04 (0.00-0.36)	0.61 (0.50-0.76)	0.18 (0.04-0.46)	0.22 (0.08-0.41)	0.60 (0.51-0.70)	0.5	1.00 (0.88-1.00)
Common effects model (rA=0.5, rc=1)	0.39 (0.24-0.50)	0.00 (0.00-0.34)	0.61 (0.18-0.26)	0.18 (0.07-0.46)	0.22 (0.10-0.41)	0.60 (0.51-0.70)	0.5	1.00
	A		**C**		**E**		**Scalar**	
Scalar Model	0.36 (0.15-0.47)		0.04 (0.00-0.19)		0.60 (0.17-0.21)		1.03 (0.97-1.09)	
	A		**C**		**E**		**r_A**	**r_C**
Homogeneity model (no sex differences)	0.35 (0.15-0.47)		0.05 (0.04-0.19)		0.60 (0.53-0.68)		0.5	1.00

[1] *Abbreviations: A= additive genetic component of variance; C=shared environmental component of variance; E=unique environmental component of variance; rA= genetic correlation, rC=shared environmental correlation, rE=non-shared environmental correlation.*

Appendix D11.1 Heterogeneity model fit statistics for the liking of non-nutritive sweetened beverages

Model	Comp.	Ep[1]	-2LL[1]	df[1]	AIC[1]	Δ-2LL	Δdf	p-value	ΔAIC
1 Saturated model		23	9537.307	2804	3929.307				
2 Full sex limitation[1]	1	9	9545.274	2818	3909.274	7.967	14	0.891	20.033
3 Full sex limitation[1]	1	9	9545.274	2818	3909.274	7.967	14	0.891	20.033
4 Common effects model[2]	2&3	8	9545.325	2819	3907.325	0.052	1	0.820	-2.00
5 Scalar Model [3]	4	6	9549.760	2821	3907.760	4.435	2	0.109	0.435
6 Homogeneity model[4]	**5**	**5**	**9551.006**	**2822**	**3907.006**	**1.246**	**1**	**0.264**	**-0.754**

[1] Model 2 & 3 = Full sex-limitation model allowing quantitative and qualitative differences between males and females and constraining either rC or rA
[2] Model 4 = Common effects model where sex-specific pathways are constrained to zero (rA=0.5, rC=1)
[3] Model 5 = Scalar model where sex-specific effects removed, but the variance components for females are all constrained to be equal to a scalar multiple of the male variance components
[4] Model 6 = Homogeneity model which assumes no sex differences

Appendix D12 Parameters estimates (95% Confidence intervals) for A, C and E for males and females considering qualitative and quantitative sex differences in the liking for fruit cordial

Model	Male			Female				
	A_m[1]	C_m[1]	E_m[1]	A_f[1]	C_f[1]	E_f[1]	r_A[1]	r_C[1]
Full sex limitation (r_A=free)	0.10 (0.36-0.36)	0.28 (0.15-0.45)	0.62 (0.18-0.78)	0.28 (0.11-0.41)	0.12 (0.03-0.25)	0.60 (0.52-0.69)	0.5 (0.00-0.50)	1.00
Full sex limitation (r_C=free)	0.38 (0.23-0.55)	0.05 (0.22-0.36)	0.57 (0.45-0.71)	0.42 (0.51-0.50)	0.00 (0.00-0.16)	0.58 (0.50-0.67)	0.5	1.00 (0.88-1.00)
Common effects model Both fixes (rA=0.5, rc=1)	0.38 (0.23-0.55)	0.05 (0.22-0.36)	0.57 (0.45-0.71)	0.42 (0.51-0.50)	0.00 (0.00-0.15)	0.58 (0.50-0.67)	0.5	1.00
	A		**C**		**E**		**Scalar**	
Scalar Model	0.32 (0.49-0.67)		0.00 (0.15-9.21)		0.68 (0.17-0.21)		-1.17 (-1.24-; -1.11)	
	A		**C**		**E**		**r_A**	**r_C**
Homogeneity model (no sex differences)	0.42 (0.36-0.49)		0.00 (0.00-0.15)		0.58 (0.52-0.64)		0.5	1.00

[1] *Abbreviations: A= additive genetic component of variance; C=shared environmental component of variance; E=unique environmental component of variance; rA= genetic correlation, rC=shared environmental correlation, rE=non-shared environmental correlation.*

Appendix D10 Heterogeneity model fit statistics for the liking of fruit cordial

Model	Comp.	Ep[1]	-2LL[1]	df[1]	AIC[1]	*Δ*-2LL	Δdf	p-value	*Δ*AIC
1 Saturated model		23	7980.13	2824	2332.13				
2 Full sex limitation	1	9	8001.96	2838	2325.96	21.829	14	0.082	-6.17
3 Full sex limitation	1	9	8001.96	2838	2325.96	21.829	14	0.082	-6.17
4 Common effects model	**2&3**	**8**	**8001.96**	**2839**	**2323.96**	**0**	**1**	**1.00**	**-2.00**
5 Scalar Model	4	6	8063.671	2841	2381.67	61.711	2	<0.001	-57.71
6 Homogeneity model	5	5	8038.078	2842	2354.08	-25.593	1	1.00	-27.59

[1] Model 2 & 3 = Full sex-limitation model allowing quantitative and qualitative differences between males and females and constraining either rC or rA

[2] Model 4 = Common effects model where sex-specific pathways are constrained to zero (rA=0.5, rC=1)

[3] Model 5 = Scalar model where sex-specific effects removed, but the variance components for females are all constrained to be equal to a scalar multiple of the male variance components

[4] Model 6 = Homogeneity model which assumes no sex differences

Appendix D13 Parameters estimates (95% Confidence intervals) for A, C and E for males and females considering qualitative and quantitative sex differences in the liking for orange juice

Model	Male			Female				
	A_m[1]	C_m[1]	E_m[1]	A_f[1]	C_f[1]	E_f[1]	r_A[1]	r_C[1]
Full sex limitation (r_A=free)	0.11 (0.00-0.27)	0.00 (0.00-0.16)	0.89 (0.73-1.00)	0.25 (0.10-0.35)	0.00 (0.00-0.10)	0.75 (0.65-0.85)	0.00 (0.00-0.50)	1.00
Full sex limitation (r_C=free)	0.07 (0.00-0.23)	0.01 (0.00-0.16)	0.92 (0.77-1.0)	0.25 (0.13-0.35)	0.00 (0.00-0.09)	0.75 (0.65-0.85)	0.5	1.00 (0.00-1.00)
Common effects model (rA=0.5, rc=1)	0.07 (0.00-0.23)	0.01 (0.00-0.16)	0.92 (0.77-1.0)	0.25 (0.13-0.35)	0.00 (0.00-0.09)	0.75 (0.65-0.85)	0.5	1.00
	A		**C**		**E**		**Scalar**	
Scalar Model	0.17 (0.08-0.25)		0.00 (0.00-0.05)		0.83 (0.75-0.91)		1.11 (1.05-1.17)	
	A		**C**		**E**		**r_A**	**r_C**
Homogeneity model (no sex differences)	0.18 (0.09-0.25)		0.00 (0.00-0.04)		0.82 (0.74-0.90)		0.5	1.00

[1] *Abbreviations: A= additive genetic component of variance; C=shared environmental component of variance; E=unique environmental component of variance; rA= genetic correlation, rC=shared environmental correlation, rE=non-shared environmental correlation.*

Appendix D10 Heterogeneity model fit statistics for the liking of orange juice

Model	Comp.	Ep[1]	-2LL[1]	df[1]	AIC[1]	Δ-2LL	Δdf	p-value	ΔAIC
1 Saturated model		23	7794.922	2826	2142.922				
2 Full sex limitation	1	9	7843.993	2840	2163.993	49.07021	14	<0.001	21.071
3 Full sex limitation	1	9	7843.993	2840	2163.993	49.07021	14	<0.001	21.071
4 Common effects model	**2&3**	**8**	**7843.993**	**2841**	**2161.993**	**0.00**	**1**	**1**	**-2.00**
5 Scalar Model	4	6	7852.797	2843	2166.797	8.804509	2	0.012	4.804
6 Homogeneity model	5	5	7866.917	2844	2178.917	14.11965	1	<0.001	12.120

[1] Model 2 & 3 = Full sex-limitation model allowing quantitative and qualitative differences between males and females and constraining either rC or rA
[2] Model 4 = Common effects model where sex-specific pathways are constrained to zero (rA=0.5, rC=1)
[3] Model 5 = Scalar model where sex-specific effects removed, but the variance components for females are all constrained to be equal to a scalar multiple of the male variance components
[4] Model 6 = Homogeneity model which assumes no sex differences

Appendix D14 Parameters estimates (95% Confidence intervals) for A, C and E for males and females considering qualitative and quantitative sex differences for the liking for milk

Model	Male			Female				
	A_m[1]	C_m[1]	E_m[1]	A_f[1]	C_f[1]	E_f[1]	r_A[1]	r_C[1]
Full sex limitation (r_A=free)	0.26 (0.07-0.41)	0.00 (0.00-0.21)	0.74 (0.59-0.90)	0.38 (0.19-0.47)	0.00 (0.10-0.32)	0.62 (0.14-0.19)	0.5 (0.30-0.50)	1.00
Full sex limitation (r_C=free)	0.14 (0.00-0.34)	0.09 (0.00-0.28)	0.78 (0.62-0.94)	0.33 (0.12-0.46)	0.04 (0.00-0.20)	0.63 (0.54-0.73)	0.5	1.00 (0.00-1.00)
Common effects model (rA=0.5, rc=1)	0.14 (0.00-0.34)	0.09 (0.00-0.28)	0.78 (0.62-0.94)	0.33 (0.17-0.46)	0.04 (0.00-0.20)	0.63 (0.54-0.72)	0.5	1.00
	A		**C**		**E**		**Scalar**	
Scalar Model	0.31 (0.22-0.39)		0.00 (0.00-0.05)		0.69 (0.61-0.77)		1.13 (1.07-1.19)	
	A		**C**		**E**		**r_A**	**r_C**
Homogeneity model (no sex differences)	0.32 (0.22-0.39)		0.00 (0.00-0.05)		0.68 (0.61-0.76)		0.5	1.00

[1] *Abbreviations: A= additive genetic component of variance; C=shared environmental component of variance; E=unique environmental component of variance; rA= genetic correlation, rC=shared environmental correlation, rE=non-shared environmental correlation.*

Appendix D14.1 Heterogeneity model fit statistics for the liking of milk

Model	Comp.	Ep[1]	-2LL[1]	df[1]	AIC[1]	Δ-2LL	Δdf	p-value	ΔAIC
1 Saturated model		23	7256.141	2684	1888.141				
2 Full sex limitation	1	9	7319.099	2698	1923.099	62.95796	14	<0.001	45.808
3 Full sex limitation	1	9	7319.099	2698	1923.099	62.95796	14	<0.001	45.808
4 Common effects model	2&3	8	7319.099	2699	1921.099	0.00	1	0.102	2.00
5 Scalar Model	**4**	**6**	**7325.083**	**2701**	**1923.083**	**5.984397**	**2**	**0.050**	**1.984**
6 Homogeneity model	5	5	7344.600	2702	1940.600	19.51656	1	<0.001	17.517

[1] Model 2 & 3 = Full sex-limitation model allowing quantitative and qualitative differences between males and females and constraining either rC or rA
[2] Model 4 = Common effects model where sex-specific pathways are constrained to zero (rA=0.5, rC=1)
[3] Model 5 = Scalar model where sex-specific effects removed, but the variance components for females are all constrained to be equal to a scalar multiple of the male variance components
[4] Model 6 = Homogeneity model which assumes no sex differences

Appendix D15 Parameters estimates (95% Confidence intervals) for A, C and E for males and females considering qualitative and quantitative sex differences for the liking for coffee

Model	Male			Female				
	A_m[1]	C_m[1]	E_m[1]	A_f[1]	C_f[1]	E_f[1]	r_A[1]	r_C[1]
Full sex limitation (r_A=free)	0.06 (0.00-0.41)	0.15 (0.04-0.34)	0.79 (0.51-0.95)	0.34 (0.12-0.46)	0.00 (0.00-0.14)	0.66 (0.53-0.81)	0.50 (0.00-0.50)	1.00
Full sex limitation (r_C=free)	0.06 (0.00-0.37)	0.15 (0.00-0.34)	0.79 (0.61-0.95)	0.34 (0.12-0.47)	0.00 (0.00-0.15)	0.66 (0.53-0.81)	0.5	1.00 (0.00-1.00)
Common effects model (rA=0.5, rc=1)	0.06 (0.00-0.37)	0.15 (0.00-0.34)	0.79 (0.61-0.95)	0.34 (0.12-0.47)	0.00 (0.00-0.14)	0.66 (0.53-0.81)	0.5	1.00
	A		**C**		**E**		**Scalar**	
Scalar Model	0.29 (0.11-0.39)		0.00 (0.00-0.06)		0.71 (0.61-0.83)		1.06 (1.00-1.14)	
	A		**C**		**E**		**r_A**	**r_C**
Homogeneity model (no sex differences)	0.29 (0.11-0.39)		0.00 (0.00-0.11)		0.71 (0.61-0.82)		0.5	1.00

[1] *Abbreviations: A= additive genetic component of variance; C=shared environmental component of variance; E=unique environmental component of variance; rA= genetic correlation, rC=shared environmental correlation, rE=non-shared environmental correlation.*

Appendix D15.1 Heterogeneity model fit statistics for the liking of SSBs

Model	Comp.	Ep[1]	-2LL[1]	df[1]	AIC[1]	*Δ*-2LL	Δdf	p-value	*Δ*AIC
1 Saturated model		23	6333.358	1882	2569.358				
2 Full sex limitation	1	9	6349.578	1896	2557.578	16.220	14	0.30	11.78
3 Full sex limitation	1	9	6349.578	1896	2557.578	16.220	14	0.30	11.78
4 Common effects model	2&3	8	6349.578	1897	2555.578	0	1	0.999	2
5 Scalar Model	4	6	6351.370	1899	2553.370	1.7917	2	0.408	2.208
6 Homogeneity model	**5**	**5**	**6355.027**	**1900**	**2555.027**	**3.6571**	**1**	**0.056**	**-1.657**

[1] Model 2 & 3 = Full sex-limitation model allowing quantitative and qualitative differences between males and females and constraining either rC or rA
[2] Model 4 = Common effects model where sex-specific pathways are constrained to zero (rA=0.5, rC=1)
[3] Model 5 = Scalar model where sex-specific effects removed, but the variance components for females are all constrained to be equal to a scalar multiple of the male variance components
[4] Model 6 = Homogeneity model which assumes no sex differences

Appendix D16 Parameters estimates (95% Confidence intervals) for A, C and E for males and females considering qualitative and quantitative sex differences for the liking for tea

Model	Male			Female				
	A_m[1]	C_m[1]	E_m[1]	A_f[1]	C_f[1]	E_f[1]	r_A[1]	r_C[1]
Full sex limitation (r_A=free)	0.42 (0.23-0.55)	0.00 (0.00-0.12)	0.58 (0.45-0.74)	0.49 (0.12-0.48)	0.00 (0.00-0.06)	0.51 (0.42-0.62)	0.02 (0.00-0.32)	1.00
Full sex limitation (r_C=free)	0.24 (0.00-0.37)	0.10 (0.06-0.44)	0.66 (0.51-0.76)	0.44 (0.28-0.48)	0.03 (0.00-0.27)	0.53 (0.43-0.69)	0.5	1.00 (0.00-1.00)
Common effects model (rA=0.5, rc=1)	0.26 (0.00-0.46)	0.09 (0.00-0.29)	0.66 (0.50-0.84)	0.45 (0.28-0.48)	0.02 (0.00-0.13)	0.53 (0.43-0.65)	0.5	1.00
	A		**C**		**E**		**Scalar**	
Scalar Model	0.41 (0.37-0.50)		0.00 (0.00-0.03)		0.59 (0.50-0.68)		1.05 (0.99-1.11)	
	A		**C**		**E**		**r_A**	**r_C**
Homogeneity model (no sex differences)	0.41 (0.32-0.50)		0.00 (0.00-0.03)		0.59 (0.50-0.68)		0.5	1.00

[1] *Abbreviations: A= additive genetic component of variance; C=shared environmental component of variance; E=unique environmental component of variance; rA= genetic correlation, rC=shared environmental correlation, rE=non-shared environmental correlation.*

Appendix D16.1 Heterogeneity model fit statistics for the liking of tea

Model	Comp.	Ep[1]	-2LL[1]	df[1]	AIC[1]	*Δ*-2LL	Δdf	p-value	*Δ*AIC
1 Saturated model		23	7087.299	2392	2303.299				
2 Full sex limitation	1	9	7130.539	2406	2318.539	43.2395	14	<0.001	-15.24
3 Full sex limitation	1	9	7130.539	2406	2318.539	43.2395	14	<0.001	11.78
4 Common effects model	2&3	8	7129.800	2407	2315.800	0.73889	1	1.00	2.739
5 Scalar Model	4	6	7136.686	2410	2316.686	2.55734	2	0.110	-0.886
6 Homogeneity model	**5**	**5**	**7134.129**	**2409**	**2316.129**	**4.32895**	**1**	**0.115**	**0.557**

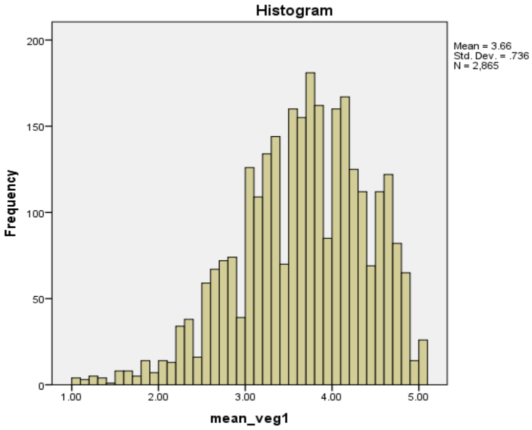
[1] Model 2 & 3 = Full sex-limitation model allowing quantitative and qualitative differences between males and females and constraining either rC or rA
[2] Model 4 = Common effects model where sex-specific pathways are constrained to zero (rA=0.5, rC=1)
[3] Model 5 = Scalar model where sex-specific effects removed, but the variance components for females are all constrained to be equal to a scalar multiple of the male variance components
[4] Model 6 = Homogeneity model which assumes no sex differences

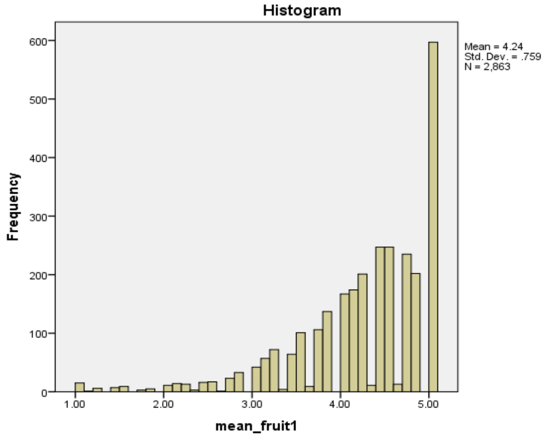
Appendix E.

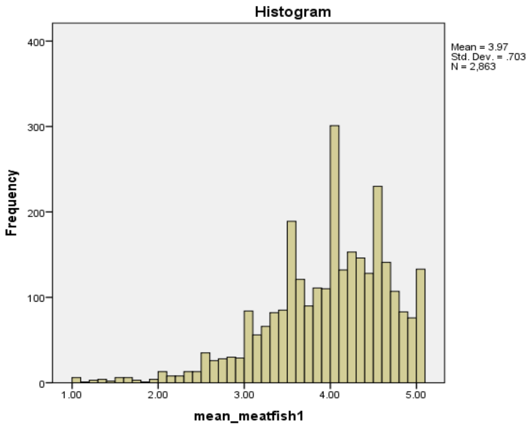
Study 3 - Cross-sectional associations of food and drink preferences and BMI in older adolescents

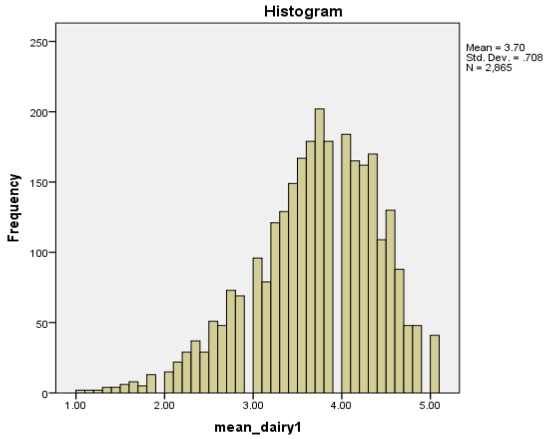
Appendix E1 Visual inspection food and drink preference score histogram to assess normality of the distribution of (A) food, and (B) drink preferences

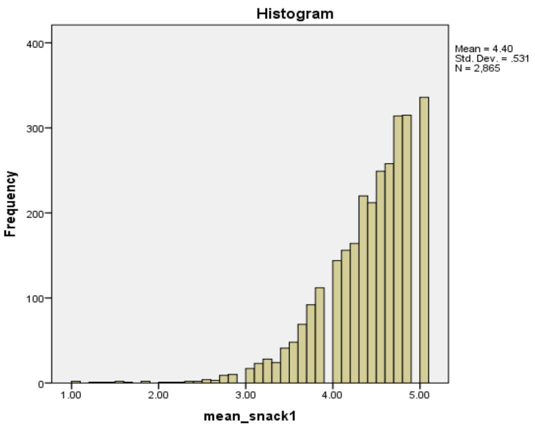
A

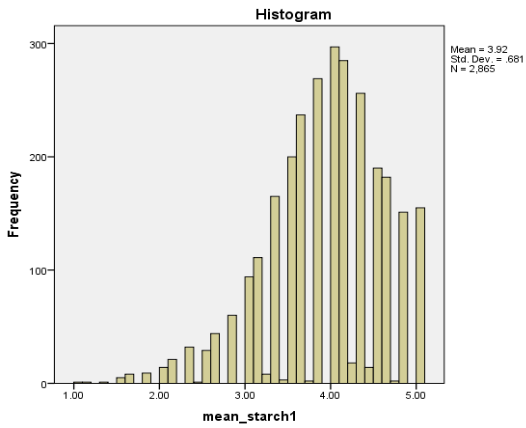




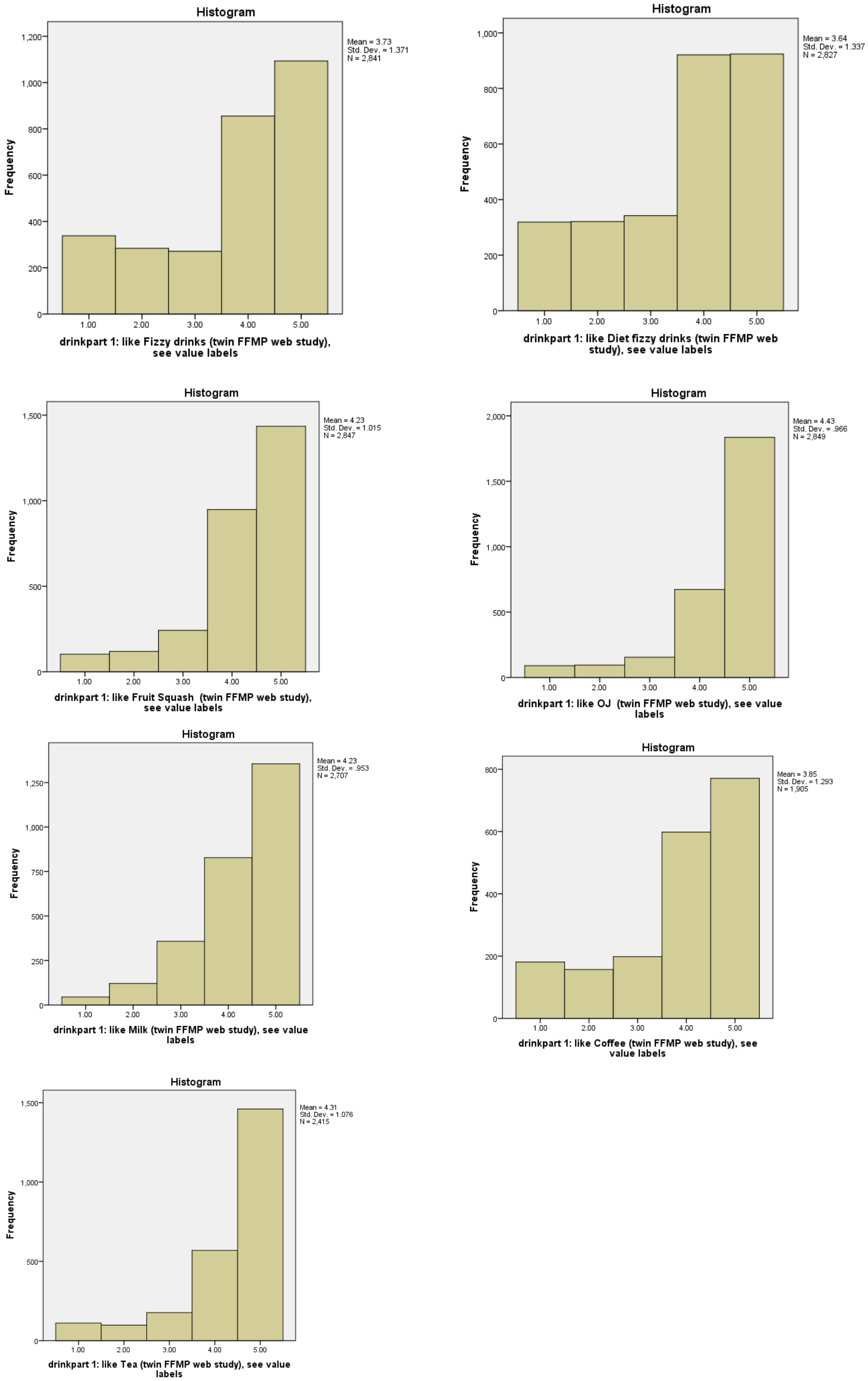






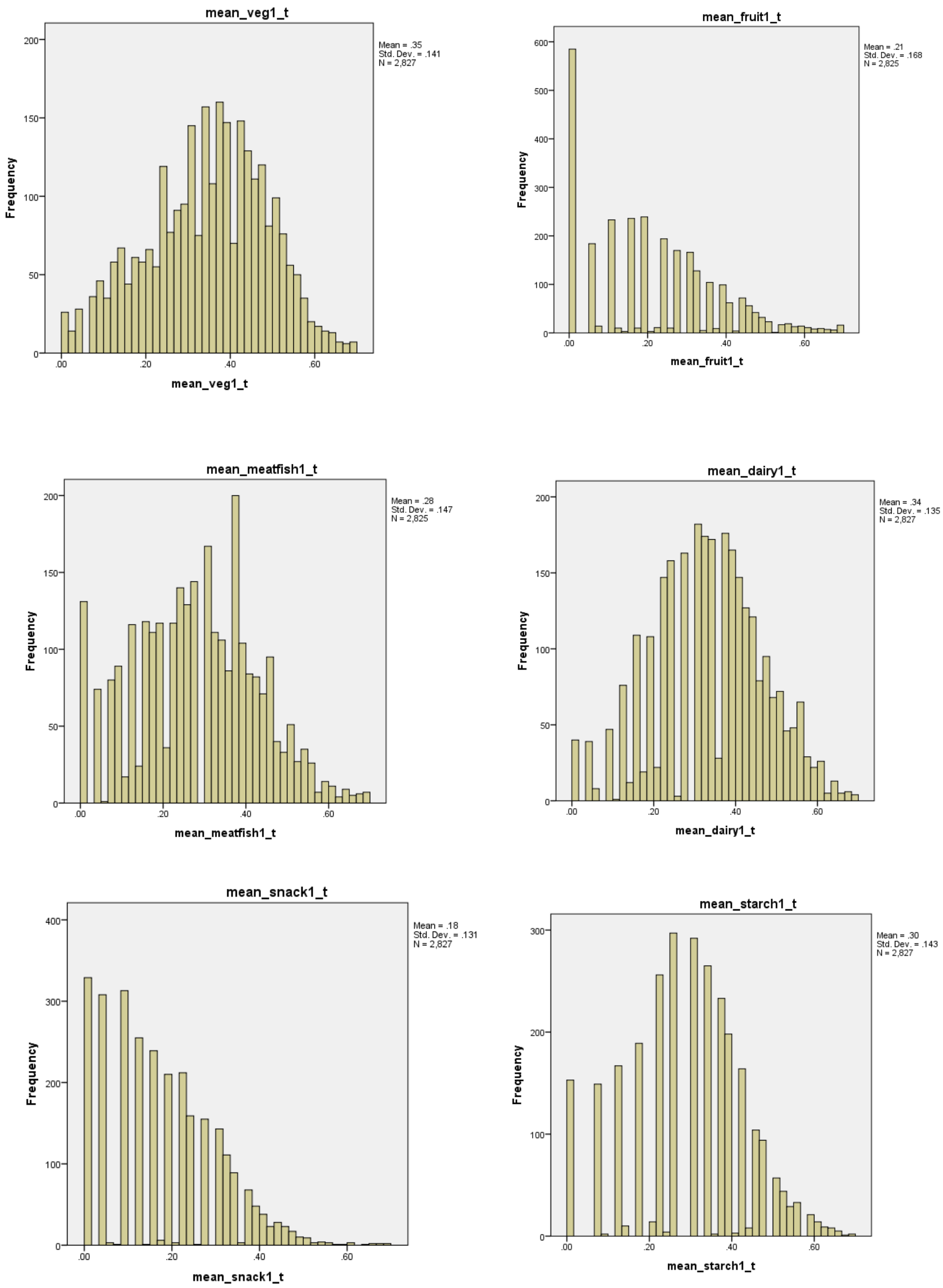


B

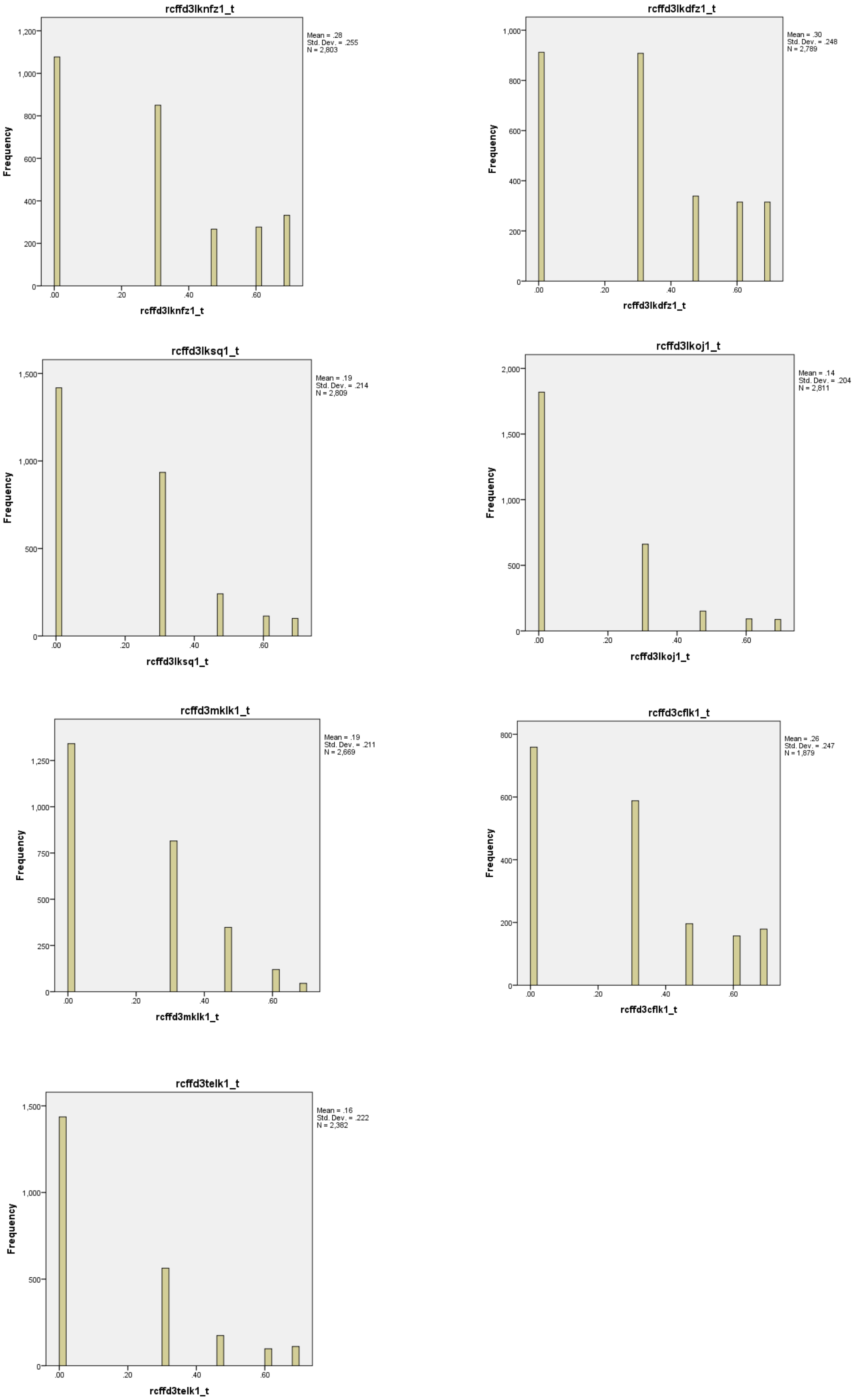

Histogram
Mean = 3.73
Std. Dev. = 1.371
N = 2,841
Frequency
drinkpart 1: like Fizzy drinks (twin FFMP web study), see value labels
Histogram
Mean = 3.64
Std. Dev. = 1.337
N = 2,827
Frequency
drinkpart 1: like Diet fizzy drinks (twin FFMP web study), see value labels
Histogram
Mean = 4.23
Std. Dev. = 1.015
N = 2,847
Frequency
drinkpart 1: like Fruit Squash (twin FFMP web study), see value labels
Histogram
Mean = 4.43
Std. Dev. = .966
N = 2,849
Frequency
drinkpart 1: like OJ (twin FFMP web study), see value labels
Histogram
Mean = 4.23
Std. Dev. = .953
N = 2,707
Frequency
drinkpart 1: like Milk (twin FFMP web study), see value labels
Histogram
Mean = 3.85
Std. Dev. = 1.293
N = 1,905
Frequency
drinkpart 1: like Coffee (twin FFMP web study), see value labels
Histogram
Mean = 4.31
Std. Dev. = 1.076
N = 2,415
Frequency
drinkpart 1: like Tea (twin FFMP web study), see value labels

Appendix E2 Visual inspection of log transformed food and drink preference score histograms to assess normality of the distribution of (A) food, and (B) drink preferences

A



B


rcffd3lknfz1_t
Mean = .28
Std. Dev. = .255
N = 2,803
Frequency
rcffd3lknfz1_t
rcffd3lkdfz1_t
Mean = .30
Std. Dev. = .248
N = 2,789
Frequency
rcffd3lkdfz1_t
rcffd3lksq1_t
Mean = .19
Std. Dev. = .214
N = 2,809
Frequency
rcffd3lksq1_t
rcffd3lkoj1_t
Mean = .14
Std. Dev. = .204
N = 2,811
Frequency
rcffd3lkoj1_t
rcffd3mklk1_t
Mean = .19
Std. Dev. = .211
N = 2,669
Frequency
rcffd3mklk1_t
rcffd3cflk1_t
Mean = .26
Std. Dev. = .247
N = 1,879
Frequency
rcffd3cflk1_t
rcffd3telk1_t
Mean = .16
Std. Dev. = .222
N = 2,382
Frequency
rcffd3telk1_t

Appendix E3 Associations of food and drink preference scores ('low' vs. 'high' likers)[1] with BMI (continuous)

	BMI			BMI[1]		
	β(SE)	p value	R^2	β(SE)	p value	R^2
Food preferences						
Vegetables	.224 (0.157)	.153	.001	.057 (.161)	.725	.013
Fruit	.453 (0.165)	.006	.003	.376 (.170)	.028	.015
Meat/Fish	.178 (0.161)	.270	<.001	.068 (.170)	.690	.013
Dairy	-.477 (0.190)	.012	.002	-.415 (.193)	.032	.015
Snacks	.268 (0.158)	.090	.001	.328 (.159)	.040	.015
Starches	.112 (0.152)	.460	<.001	.059 (.155)	.702	.013
Drink preferences						
SSBs[1]	.151 (.178)	.398	<.001	.149 (.188)	.428	.013
NNSBs[1]	-.773 (.169)	<.001	.008	-.773 (.175)	<.001	.020
Fruit squash	-.402 (.076)	.012	.002	-.251 (.165)	.128	.014
Orange juice	-.387 (.169)	.022	.002	.296 (.172)	.786	.015
Milk	.052 (.162)	.746	<.001	-.001 (.167)	.996	.013
Coffee	-.068 (.221)	.759	<.001	-.140 (.219)	.523	.017
Tea	.160 (.182)	.379	<.001	.156 (.182)	.394	.014

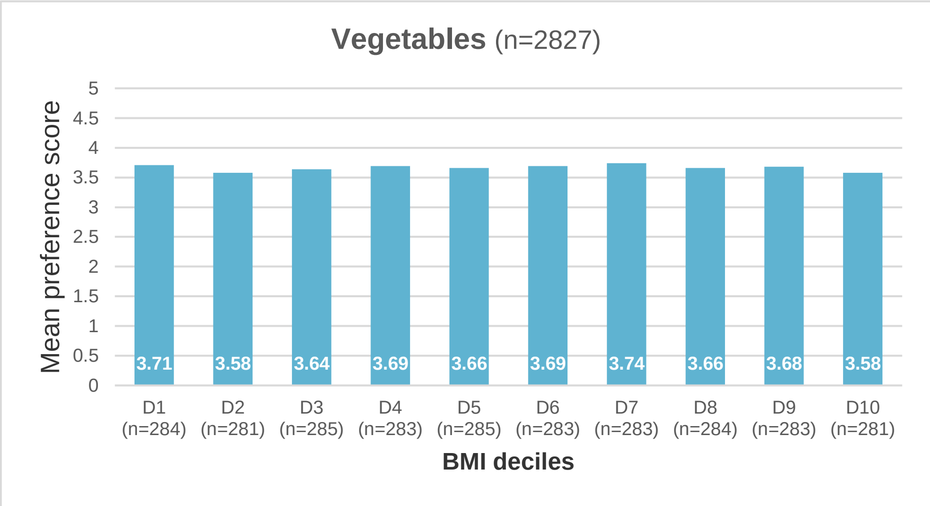
Significant findings are bolded
[1] 'High liker' defined as individuals with preferences scores above the median; set as the reference group.
[2] Model was adjusted for sex, age at questionnaire completion (years), socioeconomic status (composite scale) and ethnicity
[3] *Abbreviations: SSBs=Sugar-sweetened beverage; NNSBs= Non-nutritive sweetened beverage*

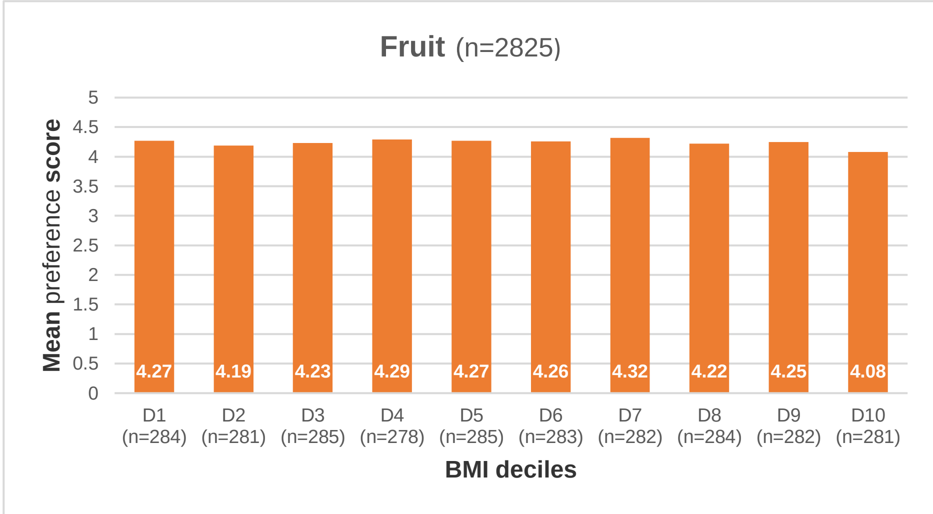
Appendix E4 Mean food and drink preference scores by BMI decile

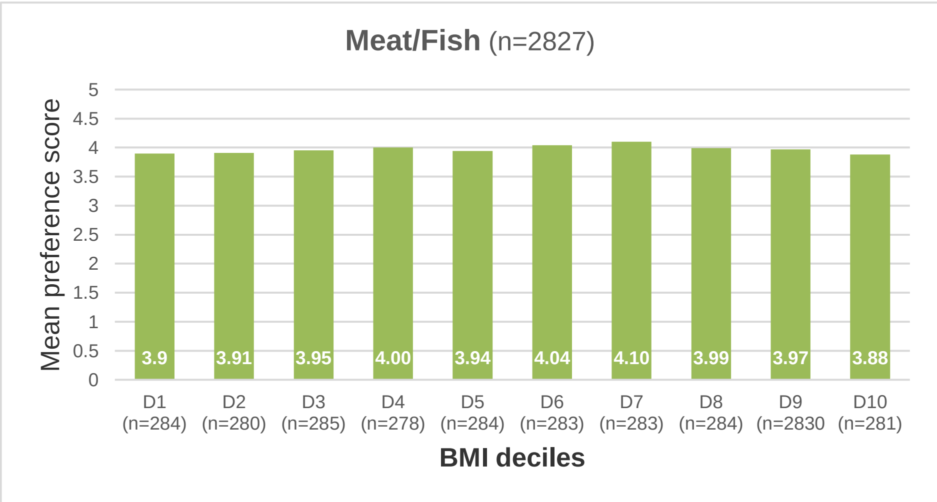
Mean score (SD)	Overall	Decile 1	Decile 2	Decile 3	Decile 4	Decile 5	Decile 6	Decile 7	Decile 8	Decile 9	Decile 10	p-value			η[2]
												Linearity	Quadratic	Cubic	
Food[1]															
Vegetables	3.66 (0.74)	3.71 (0.71)	3.58 (0.76)	3.64 (0.76)	3.69 (0.72)	3.66 (0.73)	3.69 (0.72)	3.74 (0.72)	3.66 (0.72)	3.68 (0.74)	3.58 (0.78)	.83	.11	.02	.005
Fruit	4.24 (0.76)	4.27 (0.74)	4.19 (0.79)	4.23 (0.80)	4.29 (0.78)	4.27 (0.72)	4.26 (0.74)	4.32 (0.75)	4.22 (0.76)	4.25 (0.71)	4.08 (0.81)	.17	.01	.03	.006
Meat/Fish	3.97 (0.70)	3.90 (0.74)	3.91 (0.75)	3.95 (0.71)	4.00 (0.63)	3.94 (0.71)	4.04 (0.71)	4.10 (0.61)	3.99 (0.67)	3.97(0.73)	3.88 (0.73)	.32	**<.01**	.06	.008
Dairy	3.70 (0.71)	3.66 (0.73)	3.64 (0.71)	3.71 (0.67)	3.69 (0.69)	3.64 (0.74)	3.72 (0.70)	3.76 (0.70)	3.74 (0.72)	3.74 (0.73)	3.71 (0.67)	.03	.42	.38	.003
Snacks	4.40 (0.53)	4.41 (0.57)	4.46 (0.44)	4.41 (0.56)	4.37 (0.50)	4.42 (0.50)	4.45 (0.54)	4.37(0.54)	4.42 (0.52)	4.42 (0.51)	4.29 (0.57)	.03	.10	.09	.008
Starches	3.92 (0.68)	3.93 (0.68)	3.80 (0.65)	3.91 (0.69)	3.97 (0.64)	3.91 (0.73)	3.96 (0.69)	3.97(0.64)	4.00 (0.67)	3.90 (0.67)	3.83 (0.72)	.67	**<.01**	.01	.008
Drinks[1]															
SSBs[2]	3.74 (1.37)	3.69 (1.46)	3.83 (1.33)	3.84 (1.31)	3.70 (1.31)	3.68 (1.41)	3.71 (1.37)	3.68 (1.36)	3.74 (1.33)	3.81 (1.39)	3.67 (1.41)	.55	.83	.67	.002
NNSBs[2]	3.64 (1.34)	3.48 (1.41)	3.60 (1.35)	3.57 (1.32)	3.40 (1.41)	3.55 (1.33)	3.69 (1.30)	3.58 (1.37)	3.74 (1.28)	3.85 (1.25)	3.95 (1.26)	**<.01**	**<.01**	.67	**.**014
Fruit squash	4.23 (1.02)	4.18 (1.06)	4.14 (1.02)	4.20 (1.11)	4.13 (1.02)	4.19 (1.02)	4.25 (1.01)	4.30 (0.94)	4.27(1.01)	4.34 (0.94)	4.30 (0.98)	**<.01**	.64	.28	.005
OJ	4.43 (0.97)	4.42 (0.99)	4.36 (0.98)	4.43 (0.94)	4.44 (0.87)	4.50 (0.86)	4.46 (0.94)	4.45 (0.96)	4.35 (1.07)	4.48 (0.97)	4.44 (1.03)	.61	.52	.69	.002
Milk	4.23 (0.95)	4.10 (1.03)	4.04 (1.08)	4.35 (0.89)	4.20 (0.99)	4.26 (0.93)	4.30 (0.95)	4.23 (0.95)	4.33 (0.92)	4.26 (0.89)	4.24 (0.86)	.01	.02	**.**77	.009
Coffee	3.85 (1.29)	3.74 (1.40)	3.83 (1.30)	3.98 (1.15)	3.99 (1.22)	3.82 (1.31)	3.74 (1.27)	3.80 (1.33)	3.77 (1.39)	3.92 (1.24)	3.88 (1.31)	.87	.98	.03	.005
Tea	4.31 (1.08)	4.21 (1.21)	4.25 (1.19)	4.28 (1.16)	4.37 (1.02)	4.30 (1.12)	4.38 (1.00)	4.40 (0.90)	4.36 (0.98)	4.29 (1.05)	4.24 (1.11)	.37	.20	.54	.003

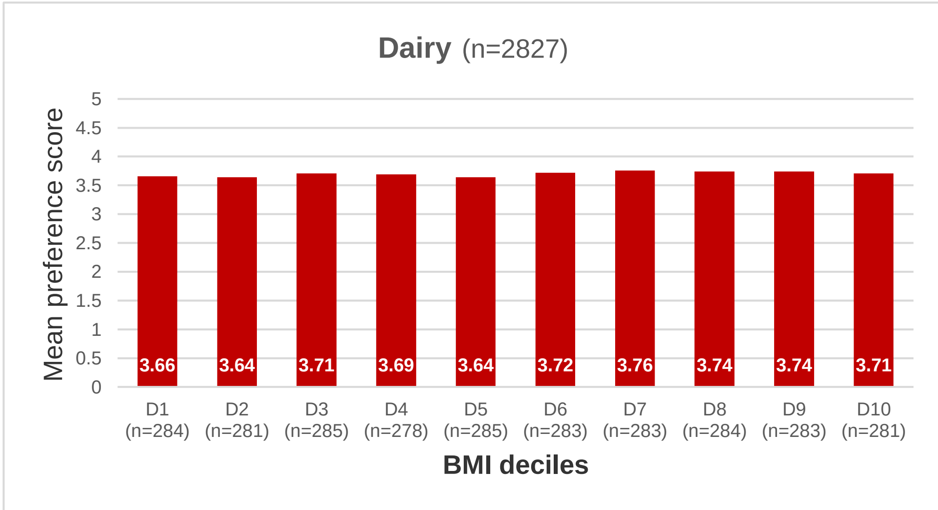
Significant findings are bolded.
Decile 1 is the lowest BMI category and Decile 10 is the highest.
[1] Sample sizes were as follows: Vegetables (n=2827), Fruit (n=2825), Meat/Fish (n=2827), Dairy (n=2827), Snacks (n=2827), Starches (n=2827) SSBs (n=2803), NNSBs (n=2789), Fruit squash (n=2809), Orange juice (n=2811), Milk (n=2669), Coffee (n=1879), Tea (n=2382)
[2] *Abbreviations: SSB=Sugar-sweetened beverage; NNSBs=Non-nutritive sweetened beverages; OJ=Orange juice*
η^2 = Eta squared; Between-Groups Sum of Squares / Total Sum of Squares. Eta squared is a measure of effect size ranging from 0-1.
Values are interpreted as ~.02=small, ~.13 =medium, ~.26=large

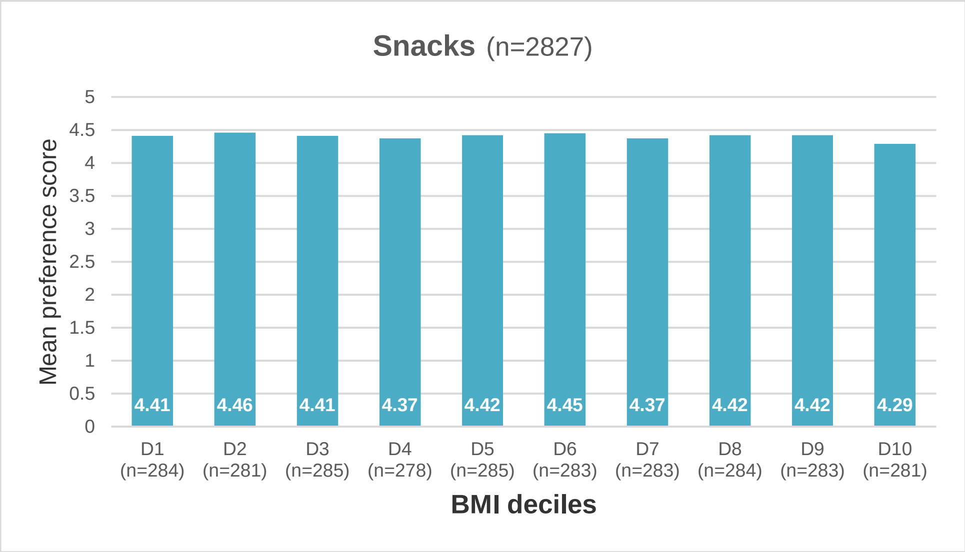
Appendix E5 Mean food preference scores by BMI decile



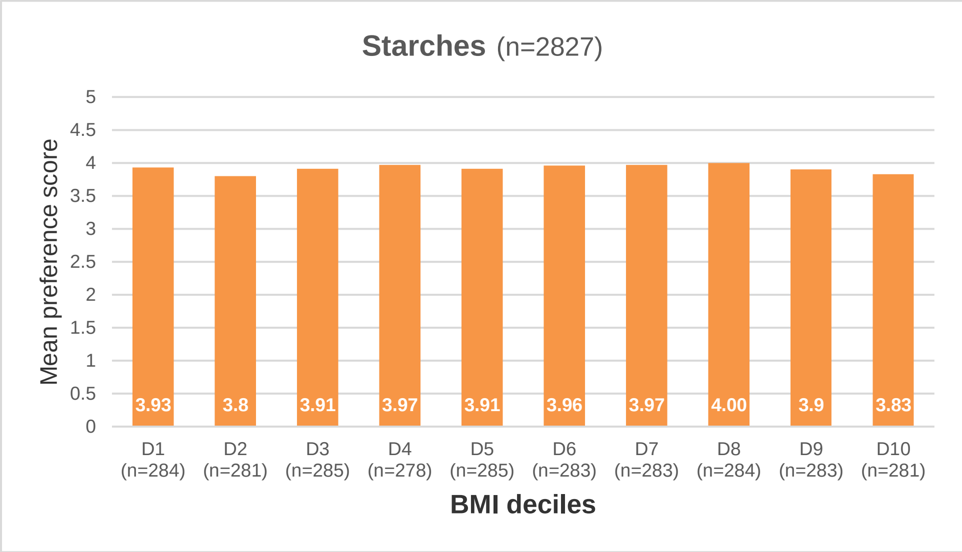






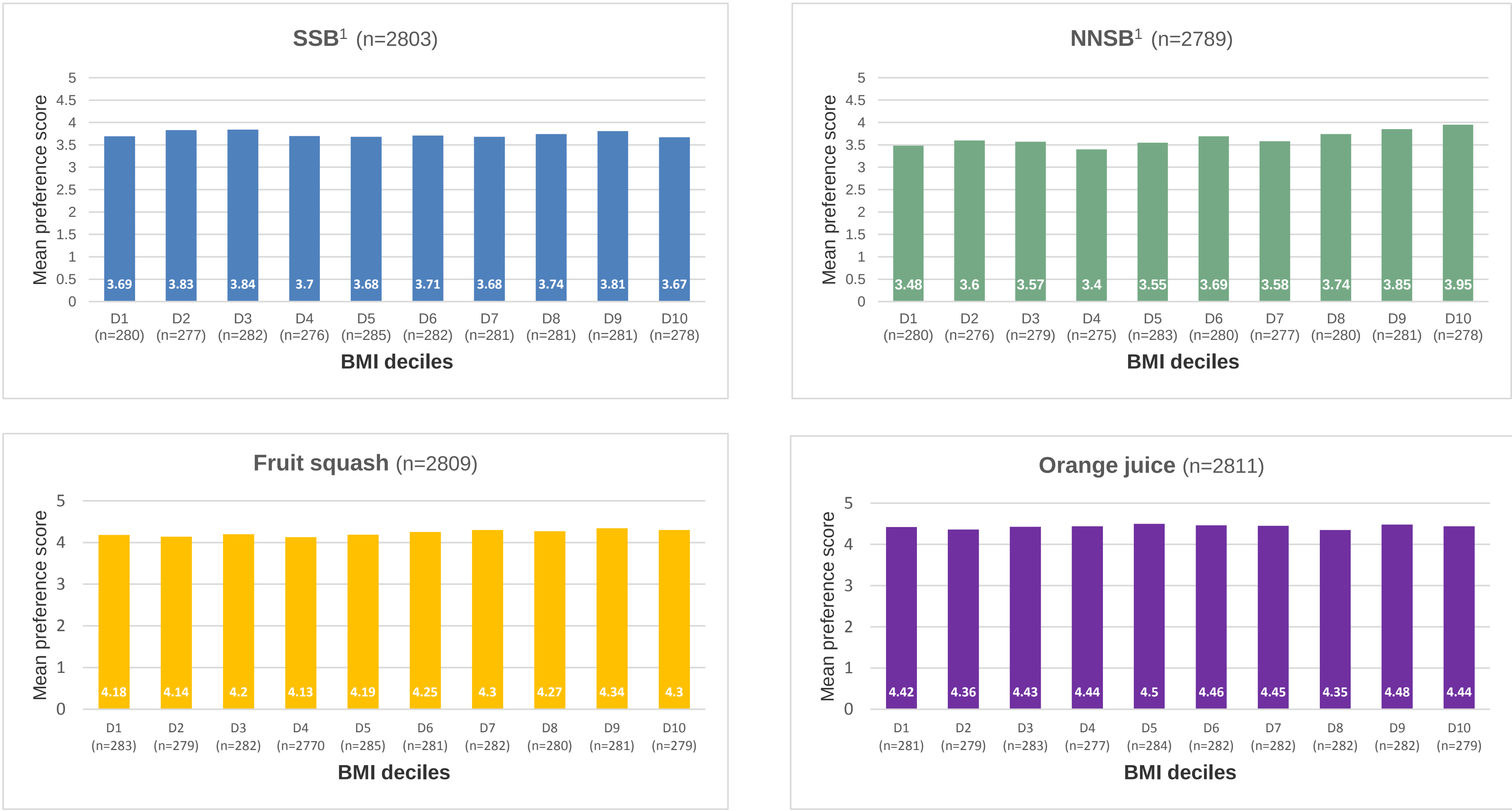


Snacks (n=2827)
Mean preference score
5
4.5
4
3.5
3
2.5
2
1.5
1
0.5
0
4.41
4.46
4.41
4.37
4.42
4.45
4.37
4.42
4.42
4.29
D1 (n=284)
D2 (n=281)
D3 (n=285)
D4 (n=278)
D5 (n=285)
D6 (n=283)
D7 (n=283)
D8 (n=284)
D9 (n=283)
D10 (n=281)
BMI deciles

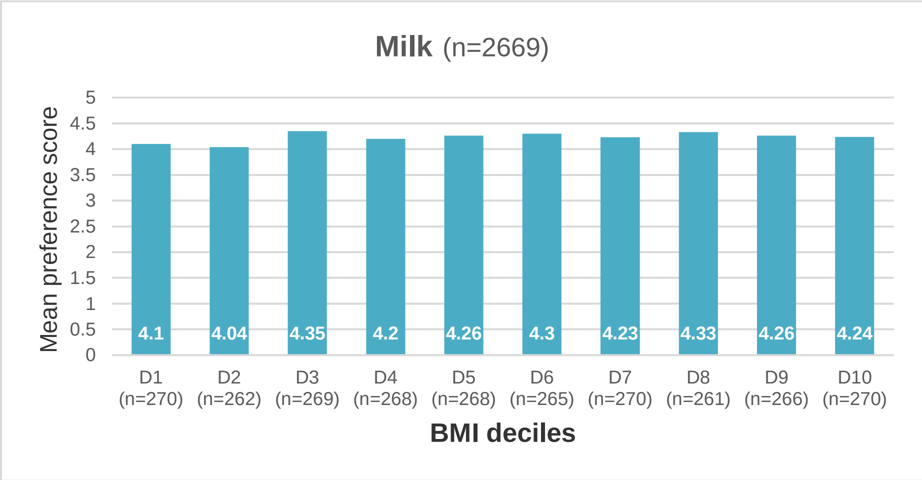


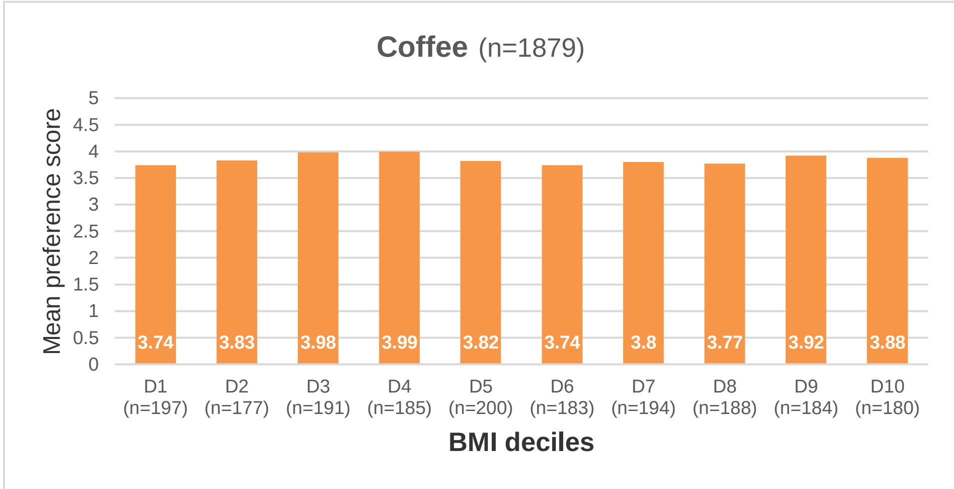
Starches (n=2827)
Mean preference score
5
4.5
4
3.5
3
2.5
2
1.5
1
0.5
0
3.93
3.8
3.91
3.97
3.91
3.96
3.97
4.00
3.9
3.83
D1 (n=284)
D2 (n=281)
D3 (n=285)
D4 (n=278)
D5 (n=285)
D6 (n=283)
D7 (n=283)
D8 (n=284)
D9 (n=283)
D10 (n=281)
BMI deciles

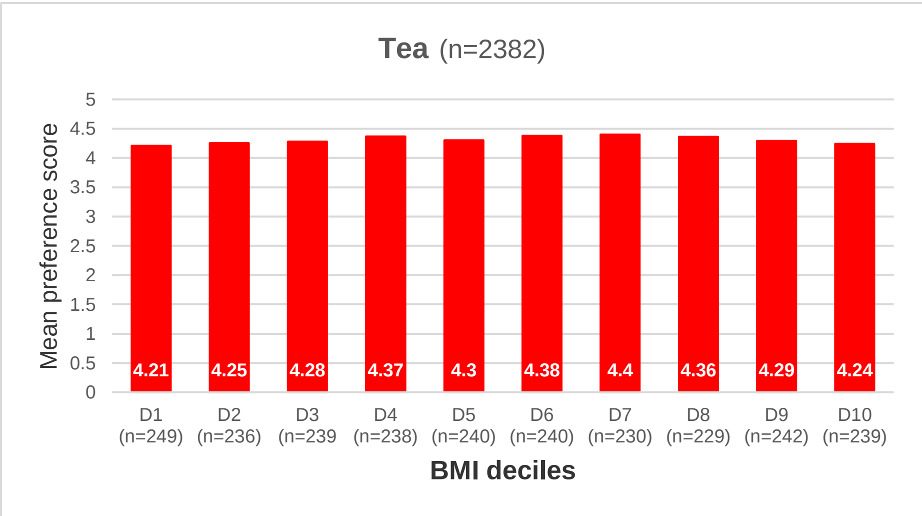
Appendix E6 Mean drink preference scores by BMI decile



[1] *Abbreviations: SSB=Sugar-sweetened beverage; NNSBs=Non-nutritive sweetened beverages*


Milk (n=2669)
Mean preference score
5
4.5
4
3.5
3
2.5
2
1.5
1
0.5
0
4.1
4.04
4.35
4.2
4.26
4.3
4.23
4.33
4.26
4.24
D1 (n=270)
D2 (n=262)
D3 (n=269)
D4 (n=268)
D5 (n=268)
D6 (n=265)
D7 (n=270)
D8 (n=261)
D9 (n=266)
D10 (n=270)
BMI deciles


Coffee (n=1879)
Mean preference score
5
4.5
4
3.5
3
2.5
2
1.5
1
0.5
0
3.74
3.83
3.98
3.99
3.82
3.74
3.8
3.77
3.92
3.88
D1 (n=197)
D2 (n=177)
D3 (n=191)
D4 (n=185)
D5 (n=200)
D6 (n=183)
D7 (n=194)
D8 (n=188)
D9 (n=184)
D10 (n=180)
BMI deciles


Tea (n=2382)
Mean preference score
5
4.5
4
3.5
3
2.5
2
1.5
1
0.5
0
4.21
4.25
4.28
4.37
4.3
4.38
4.4
4.36
4.29
4.24
D1 (n=249)
D2 (n=236)
D3 (n=239
D4 (n=238)
D5 (n=240)
D6 (n=240)
D7 (n=230)
D8 (n=229)
D9 (n=242)
D10 (n=239)
BMI deciles

Appendix F.

Chapter 7 - Sweetness preference modification intervention in relation to hot beverages in young adults: The REduction of Sugar In tea Study (RESIST)

Appendix F1 Recruitment message featured in UCL newsletter

Take part in a study examining how to give up sugar in your tea

7 December 2016

Are you looking to make some healthy changes in the new year?

Most people generally like sweet tastes but would like to consume less added sugar because of the bad effects it may have on general health, weight or their teeth. A lot of interest has been directed at reducing the consumption of sugar from soft drinks, but sweetened coffees and teas are often underestimated in contributing to high levels of sugar intake.

Little is known on how people can best decrease their liking of sweetness in drinks and for this reason, researchers are looking to investigate different strategies to stop taking sugar in your tea.

The study will be guided via email and a mobile phone app, by researchers at the Health Behaviour Centre (Department of Epidemiology and Public Health, UCL).

What's in it for me?

- All participants will be reimbursed for their time with **£10 Amazon vouchers.**
- You will receive guidance and tips on how to reduce your sugar intake in tea
- You will be given the opportunity to opt-in to further interview studies, rewarded with a **further £10 Amazon** voucher

Who?

- Students at UCL who regularly drink tea (sweetened with sugar)
- Have an Android phone or an iPhone.

What?

- The study will start in January 2017
- You will be asked to fill in two questionnaires; one at the start and one at the end of the study.
- You will need to download and install an app and use it for a period of a month.
- The app requires minimal user input (less than 3 minutes per day).

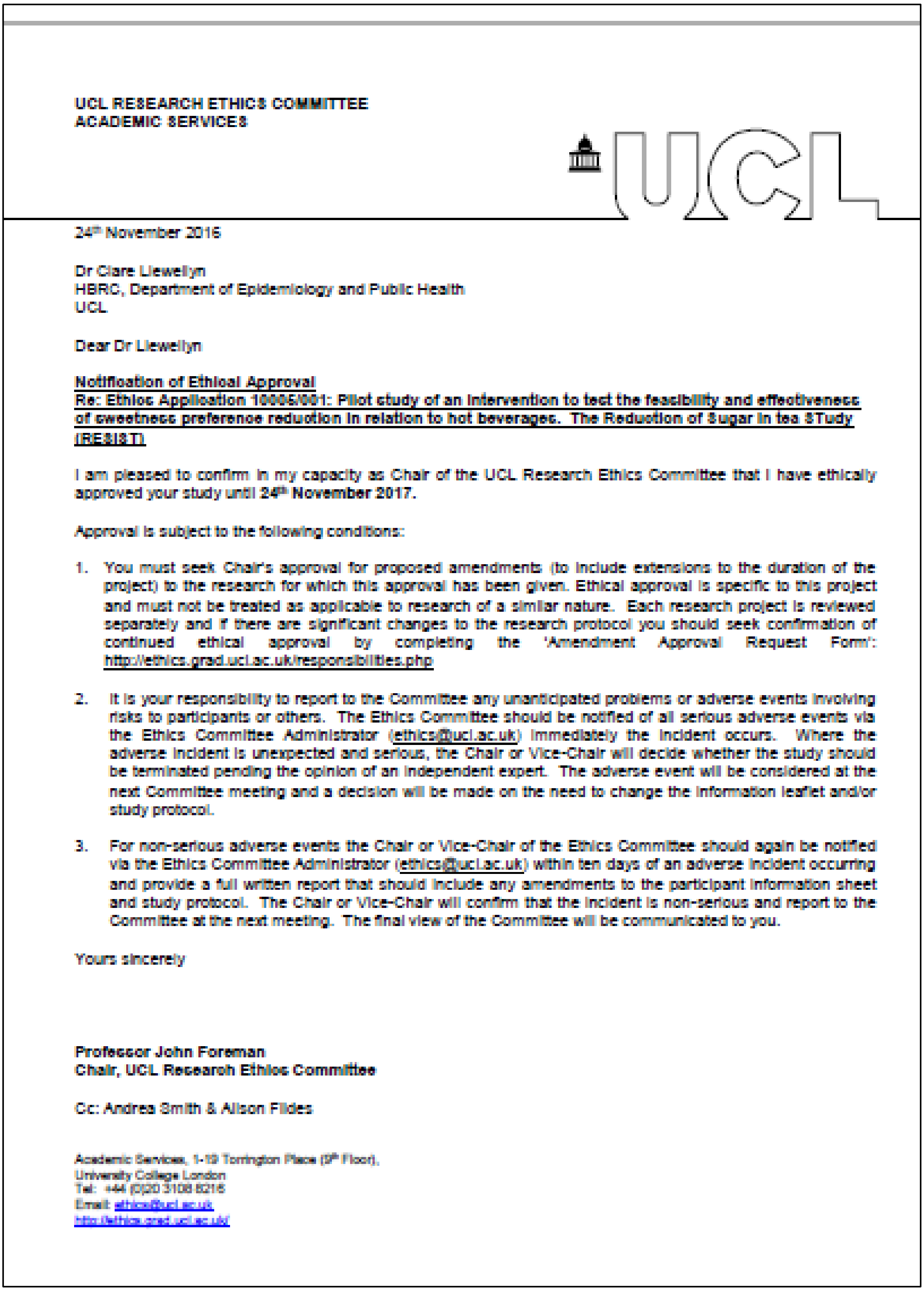
To register your interest, please complete this short form.

For any questions please contact the student researcher Andrea Smith.

This project has been approved by UCL ethics: Project reference 10005/001

Andrea Smith, UCL Epidemiology and Public Health

Appendix F2 UCL Ethics Committee approval for RESIST



UCL RESEARCH ETHICS COMMITTEE
ACADEMIC SERVICES

24[th] November 2016

Dr Clare Llewellyn
HBRC, Department of Epidemiology and Public Health
UCL

Dear Dr Llewellyn

Notification of Ethical Approval
Re: Ethics Application 10005/001: Pilot study of an intervention to test the feasibility and effectiveness of sweetness preference reduction in relation to hot beverages. The Reduction of Sugar in tea STudy (RESIST)

I am pleased to confirm in my capacity as Chair of the UCL Research Ethics Committee that I have ethically approved your study until **24[th] November 2017.**

Approval is subject to the following conditions:

1. You must seek Chair's approval for proposed amendments (to include extensions to the duration of the project) to the research for which this approval has been given. Ethical approval is specific to this project and must not be treated as applicable to research of a similar nature. Each research project is reviewed separately and if there are significant changes to the research protocol you should seek confirmation of continued ethical approval by completing the 'Amendment Approval Request Form': http://ethics.grad.ucl.ac.uk/responsibilities.php

2. It is your responsibility to report to the Committee any unanticipated problems or adverse events involving risks to participants or others. The Ethics Committee should be notified of all serious adverse events via the Ethics Committee Administrator (ethics@ucl.ac.uk) immediately the incident occurs. Where the adverse incident is unexpected and serious, the Chair or Vice-Chair will decide whether the study should be terminated pending the opinion of an independent expert. The adverse event will be considered at the next Committee meeting and a decision will be made on the need to change the information leaflet and/or study protocol.

3. For non-serious adverse events the Chair or Vice-Chair of the Ethics Committee should again be notified via the Ethics Committee Administrator (ethics@ucl.ac.uk) within ten days of an adverse incident occurring and provide a full written report that should include any amendments to the participant information sheet and study protocol. The Chair or Vice-Chair will confirm that the incident is non-serious and report to the Committee at the next meeting. The final view of the Committee will be communicated to you.

Yours sincerely

Professor John Foreman
Chair, UCL Research Ethics Committee

Cc: Andrea Smith & Alison Fildes

Academic Services, 1-19 Torrington Place (9[th] Floor),
University College London
Tel: +44 (0)20 3108 8216
Email: ethics@ucl.ac.uk
http://ethics.grad.ucl.ac.uk/

Appendix F3 RESIST instruction booklet; Gradual reduction condition

UCL

The REduction of Sugar In tea Study

RESIST

INSTRUCTION BOOKLET

Thank you for participating in the RESIST study.

This booklet contains all the information you will need throughout the 4 weeks of RESIST.

We suggest that you start by reading the entire booklet, and we encourage you to **print a copy** so that you can make notes on the pages. We have included a number of handy tips to help you get started and to keep you on track with your goal of reducing the amount of added sweetness in your tea.

A guide on how to install the PACO app on your phone can be found in the last section of this booklet. This app will be sending you daily notifications to complete a short daily questionnaire.

Don't worry if this is intimidating to begin with, the longer you will stick to your sugar reduction protocol, the easier it will become. Even if you forget to complete your PACO app logging task on some days, we would still like you to **complete it as often as possible** (irrespective of whether you manage to stick to your sugar reduction schedule).

If you have any questions, please contact Andrea Smith:
E-mail: andrea.smith.14@ucl.ac.uk
Telephone: 020 7679 1736

In this booklet you will find:

TIPS TO REDUCE & TRACK SUGAR IN TEA

- When you start to reduce sugar in tea, it is likely that for the first 3-5 days you might feel like your tea has no taste or you might not enjoy it as much. But you'll get used to it very quickly once you start to taste the 'real' aroma of tea and this can be very pleasant.
- Remove the sugar you keep close to your tea bags. Alternatively, put it away in a different cupboard to avoid automatically adding the sugar.
- If anybody offers you a cup of tea, let them know that you don't take sugar any more. If people question your new behaviour, have a response ready, e.g. "I prefer it this way", or "I am making healthy changes".
- Tell your friends and family about your intention to reduce the amount of sugar in your tea; group support will encourage you to stick it out.
- Reward yourself once a week for sticking to your schedule. Mini rewards could include:
  - Buying yourself a fancy new mug or tea to-go container
  - Buy an interesting new tea that you have always wanted to try
  - Post about your successes on Twitter, Facebook or any other social media
  - Plan a night out with your friends
  - Add £1 to a coin jar for every day you stick to your protocol – the growing collection of money will be a visual reminder of what you've accomplished and will motivate you to add to it
  - Treat yourself to a magazine

HEALTH BENEFITS OF REDUCING SUGAR INTAKE

TIPS TO TRACK SUGAR IN TEA

For the purpose of this study, we would like you to initially track and reduce **ANY** added sweetness to your beverages. This includes white sugar, brown sugar, flavoured syrups, honey, agave syrup and/or any artificial sweeteners.

When measuring one teaspoon of sugar, please ensure that it is NOT heaped.

1 teaspoon of sugar = 4 grams

= 3 - 4 grams of sugar (average)

1 pump of flavoured syrup

= 5 grams of sugar (average)

YOUR RESIST SUGAR SCHEDULE [GR]

You will be gradually eliminating sweetness in tea

To get you started, please install the smartphone app 'PACO' https://www.pacoapp.com.

Detailed installation guidelines can be found at the end of this booklet. To use the app, you will have to use your allocated Gmail account (e.g. 2017resist.XX@gmail.com) which will be sent to you in an e-mail

The study will consist of 3 main phases:

(1) **Warm-up phase (starting on Monday 6/2):** Maintain your usual eating/drinking behaviours for 2 days but start using the RESIST smartphone app to track your tea liking, and the amount of sugar that you add to your tea on an average day.

(2) **Start of the intervention schedule (Wednesday 8/2):** During this 1-month period you will be given specific instructions on how to cut-back on the amount of sugar in your tea. We ask you to complete a daily questionnaire via the app during this phase.

(3) **Completion phase:** At the end of the study, you will complete a 5-minute questionnaire sent to you via e-mail. Following this, you will receive a further 5-min follow-up questionnaire a couple of weeks after the study has been completed. Once this final questionnaire has been returned, then you will receive your Amazon voucher.

YOUR RESIST SUGAR SCHEDULE [GR]

- On Sunday the 5/2 you will receive an e-mail to let you know that the RESIST 'warm-up' phase is about to start.

DON'T FORGET

You will have received a set of measuring spoons that will help you to track precisely how much sugar you add to a typical cup.

- You will then be sent a further e-mail to signal the start of the RESIST schedule (**Wednesday the 8/2**). From this day onwards (Day 1), we ask you to **gradually reduce adding sugar, honey or artificial sweeteners to your tea.** This might be quite challenging at first but research suggests that this gradual approach is an effective way to decrease your preference for sweetness in tea.
- To help you in following a personal gradual reduction protocol, we have sent you a schedule that maps out by how much and when you need to decrease the amount of sugar that you add to your tea. **You will receive personalized e-mail reminders to signal the start of the next reduction step** (see the next page).
- The app on your phone will send you a daily questionnaire every evening asking you 4 short questions on your liking of the sweetness level of your tea for that day. Please take the time to complete this 2-minute task every day.
- At the end of the study period we will be sending you a follow-up questionnaire to ask you about your general experience of the study. Shortly afterwards you will receive a £10 Amazon voucher as a thank you for your participation.

Number of teaspoons of sugar in my tea at the start of the study ___

YOUR RESIST SUGAR SCHEDULE [GR]

Gradual sweetness reduction schedule

	Date	Day	Reduction of sugar
Step 1	**8/2**	**1→ E-mail Reminder on previous day**	**Reduction to 75%**
	9/2	2	75%
	10/2	3	75%
	11/2	4	75%
	12/2	5	75%
	13/2	6	75%
	14/2	7	75%
	15/2	8 → E-mail reminder	75%
Step 2	**16/2**	**9**	**Reduction to 50%**
	17/2	10	50%
	18/2	11	50%
	19/2	12	50%
	20/2	13	50%
	21/2	14	50%
	22/2	15	50%
	23/2	16→ E-mail reminder	50%
Step 3	**24/2**	**17**	**Reduction to 25%**
	25/2	18	25%
	26/2	19	25%
	27/2	20	25%
	28/2	21	25%
	1/3	22	25%
	2/3	23→ E-mail Reminder	25%
Step 4	**3/3**	**24**	**Reduction to 0%**
	4/3	25	0%
	5/3	26	0%
	6/3	27	0%
	7/3	28	0%
	8/3	29	0%
	9/3	30	0%
	10/3	31	0%
FINISHED!			

If starting level is 1 teaspoon of sugar per cup:

75% = ¾ teaspoons of sugar
50% = ½ teaspoons of sugar
25% = ¼ teaspoons of sugar

If starting level is 2 teaspoons of sugar per cup:

75% = 1.5 teaspoons of sugar
50% = 1 teaspoons of sugar
25% = 1/2 teaspoons of sugar

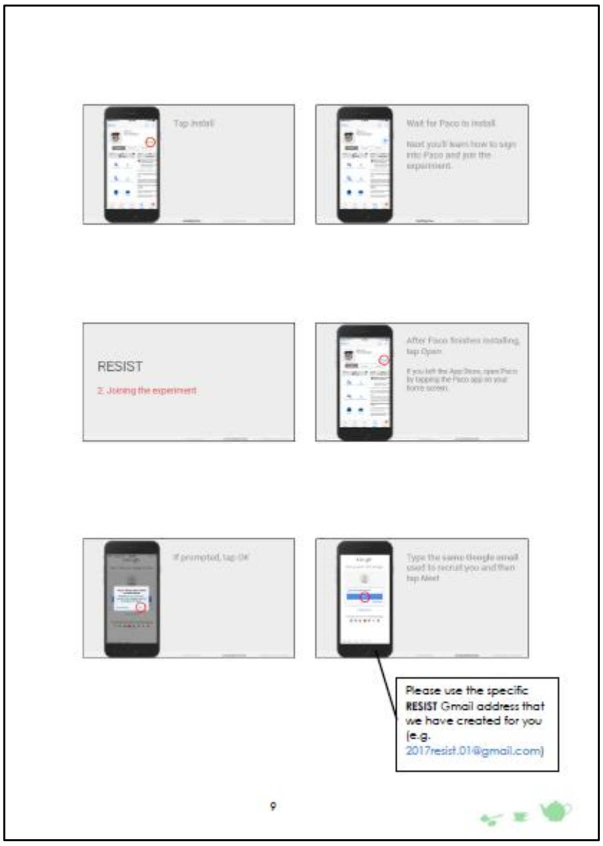
If starting level is 3 teaspoons of sugar per cup:

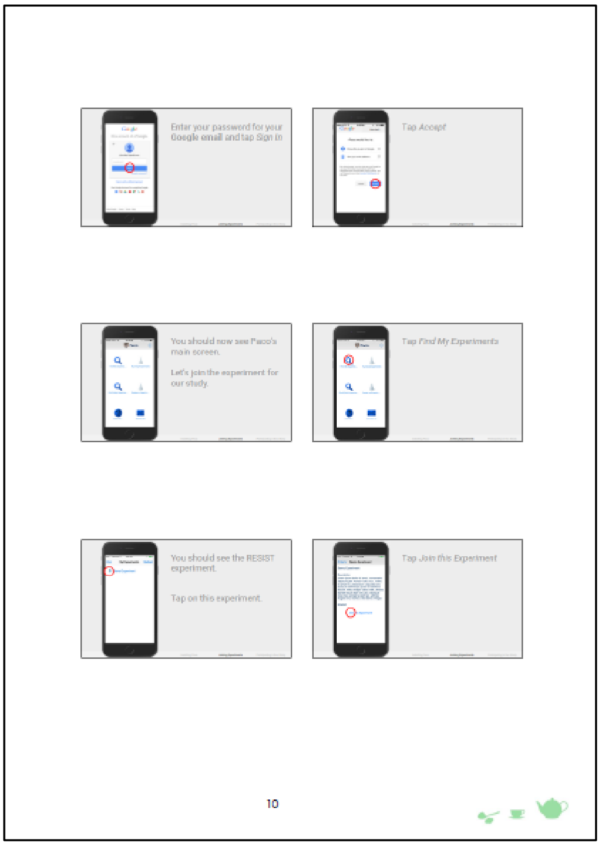
75% = 2 ¼ teaspoons of sugar
50% = 1.5 teaspoons of sugar
25% = ¾ teaspoons of sugar

INSTALLATION GUIDES FOR PACO

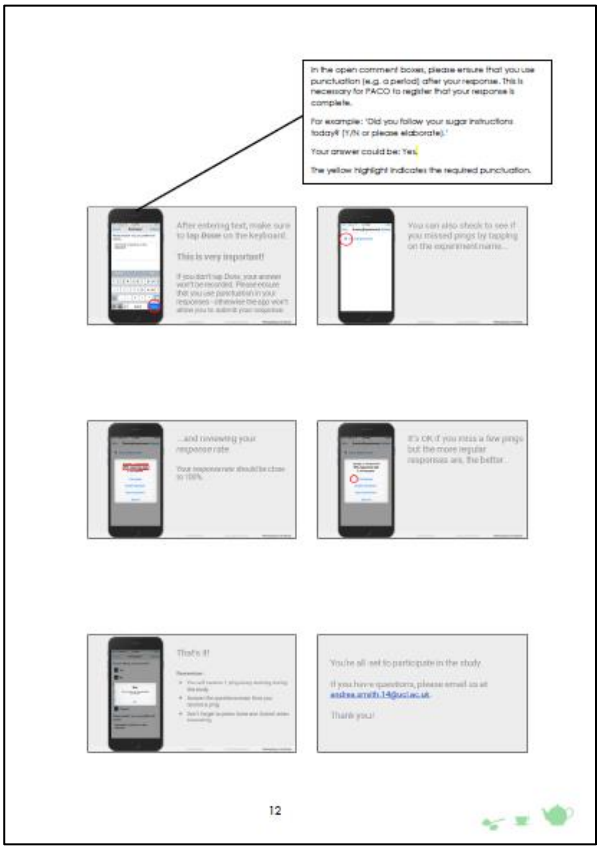
PACO is the smartphone application that will be assisting you throughout the study.

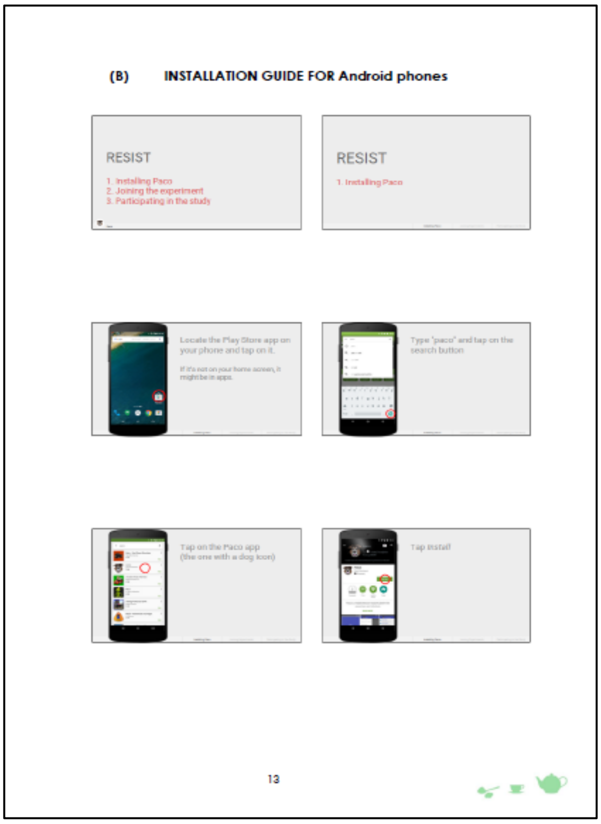
(A) INSTALLATION GUIDE FOR iOS phones

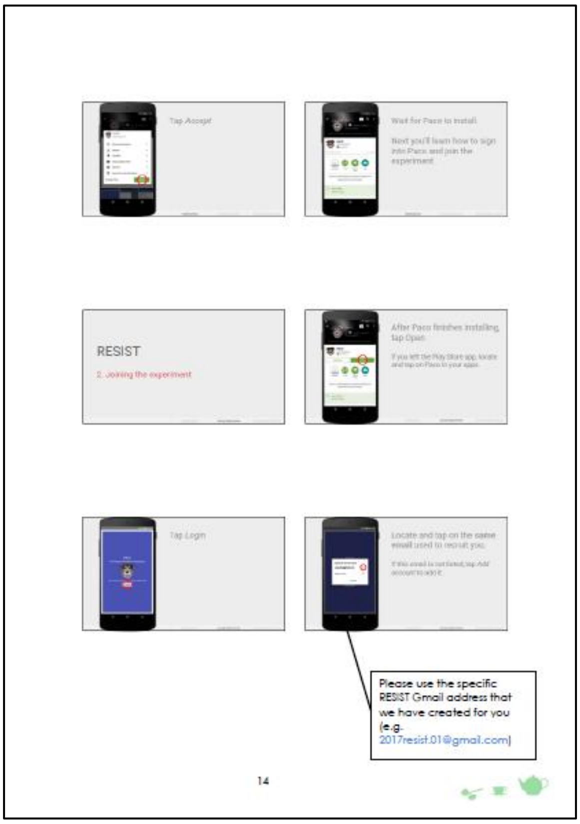

Tap Install
Wait for Paco to install.
Next you'll learn how to sign into Paco and join the experiment.
RESIST
2. Joining the experiment
After Paco finishes installing, tap Open
If prompted, tap OK
Type the same Google email used to recruit you and then tap Next
Please use the specific RESIST Gmail address that we have created for you (e.g. 2017resist.01@gmail.com)
9

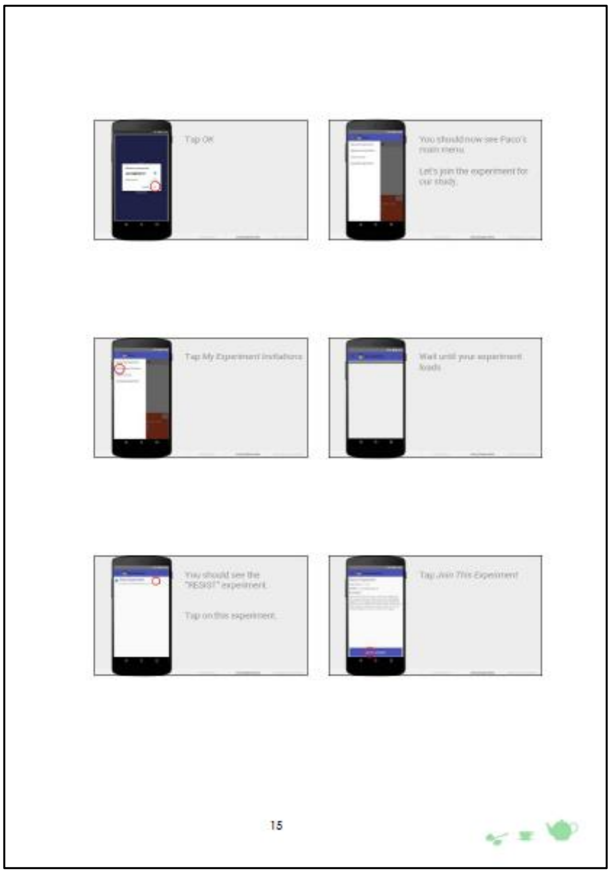

Enter your password for your Google email and tap Sign in
Tap Accept
You should now see Paco's main screen.
Let's join the experiment for our study.
Tap Find My Experiments
You should see the RESIST experiment.
Tap on this experiment.
Tap Join this Experiment
10

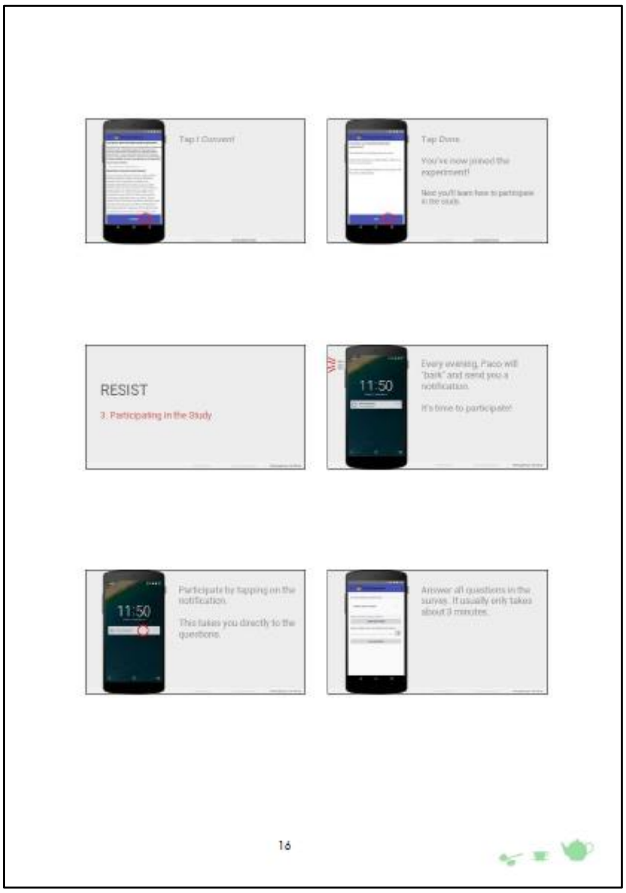

Tap I Consent
You've now joined the experiment!
Next you'll learn how to participate in the study.
RESIST
3. Participating in the Study
Every evening, Paco will "bark" and send you a notification.
It's time to participate!
Participate by swiping the notification.
This takes you directly to the questions.
Answer all questions in the survey. It usually only takes about 3 minutes.
11

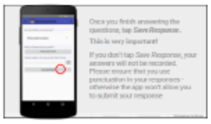

In the open comment boxes, please ensure that you use punctuation (e.g. a period) after your response. This is necessary for PACO to register that your response is complete.
For example: 'Did you follow your sugar instructions today? (Y/N or please elaborate).'
Your answer could be: Yes.
The yellow highlight indicates the required punctuation.
After entering text, make sure to tap Done on the keyboard!
This is very important!
You can also check to see if you missed pings by tapping on the experiment name...
...and reviewing your response rate
Your response rate should be close to 100%.
It's OK if you miss a few pings but the more regular responses are, the better.
That's it!
You're all set to participate in the study
If you have questions, please email us at andrea.smith.14@ucl.ac.uk.
Thank you!
12


(B) INSTALLATION GUIDE FOR Android phones
RESIST
1. Installing Paco
2. Joining the experiment
3. Participating in the study
RESIST
1. Installing Paco
Locate the Play Store app on your phone and tap on it.
If it's not on your home screen, it might be in apps.
Type "paco" and tap on the search button
Tap on the Paco app (the one with a dog icon)
Tap Install
13


Tap Accept
Wait for Paco to install.
Next you'll learn how to sign into Paco and join the experiment
RESIST
2. Joining the experiment
After Paco finishes installing, tap Open.
If you left the Play Store app, locate and tap on Paco in your apps.
Tap Login
Locate and tap on the same email used to recruit you.
If this email is not listed, tap Add account to add it.
Please use the specific RESIST Gmail address that we have created for you (e.g. 2017resist.01@gmail.com)
14


Tap OK
You should now see Paco's main menu.
Let's join the experiment for our study.
Tap My Experiment Invitations
Wait until your experiment loads
You should see the "RESIST" experiment.
Tap on this experiment.
Tap Join This Experiment
15


Tap I Consent
Tap Done
You've now joined the experiment!
Next you'll learn how to participate in the study.
RESIST
3. Participating in the Study
11:50
Every evening, Paco will "bark" and send you a notification.
It's time to participate!
11:50
Participate by tapping on the notification.
This takes you directly to the questions.
Answer all questions in the survey. It usually only takes about 3 minutes.
16



Extra note for Android users:

When you are prompted to complete your daily drink preference questions on your smartphone in PACO, you will see that there is an option to dictate your response by pressing the small Microphone logo in PACO. Please feel free to use this option if this makes the process quicker or more fun to complete for you.

FREQUENTLY ASKED QUESTIONS

(1) What is PACO?

PACO is an open-source platform for behavioral research. It includes a web interface and mobile apps (for both Android and iOS). Since the PACO app travels with participants throughout the day on their smartphones, it can operate at meaningful moments to log data (e.g., answer research questions).

(2) Does PACO require a lot of resources from the phone?

No, PACO's memory usage is minimal (typically less than 5MB).

(3) Do I have to use the assigned Gmail to login to the PACO app?

For the purpose of this study we have created individualized Gmail accounts for each participant. This will allow us to keep track of who is who in the study and will keep your private Gmail account completely private. We will not be using the assigned e-mail addresses to send out any communications. Correspondence will be strictly sent to your own account. If at any point you forget your assigned Gmail account name or password, please just send an e-mail (andrea.smith.14@ucl.ac.uk) and we will send it to you as soon as possible.

(4) When will I receive my voucher?

Once you have completed the 1-month follow-up questionnaire, then we will send you a £10 Amazon voucher as a thank you for your participation.

(5) I have questions about PACO or the study. How do I get help?

Send an e-mail to andrea.smith.14@ucl.ac.uk and somebody from the RESIST team will respond rapidly

(6) When and where are the experimental tasting sessions taking place? And why are you undertaking these in-person assessments?

The assessment sessions will be at baseline (at the start) and after the 4-week intervention period. The short 5-minute test will measure your objective liking for sweetness. The sessions will be taking place in a booked private room in the UCL building 1-19 Torrington Place. Your participation in this short voluntary additional activity will be rewarded with an additional £10 for attendance at both experimental sessions.

(7) When and where are the interviews taking place? And why are you interested in interviewing me?

The follow-up interviews will be taking place after the 4-week intervention period. We estimate that each interview will take around 20 minutes. The interviews will be taking place in a booked private room in the UCL building 1-19 Torrington Place. Alternatively, if it is any easier for you, then we can arrange for the interviews to be done over the phone. The aim of the interviews is to understand your opinions and general thoughts about the intervention. Your participation in this short voluntary additional activity will be rewarded with an additional £10 for the one interview.

(8) How are you storing my data? Is my data secure?

All data collected throughout the study will be stored in a password secured database. Access to this database is limited to research staff only. Data will be stored in compliance with the 1998 Data Protection Act. In short, all steps to ensure that your data is stored safely have been implemented.

(9) I'm really busy and stressed and I no longer have the time participate in the study. How do I contact the research team to let them know about my decision?

We completely understand that something may come up and that you no longer are able to participate in this research study. You are free to withdraw from the study at any time without giving a reason. The easiest manner is to send us an e-mail to let us know that you have to withdraw from the study. That's it!

(10) Oops, I've been forgetting to complete my daily sweetness preference questionnaire recently. Is this a big problem?

That's no problem, just try to get back into the habit of completing your daily questionnaire. The more detailed your response data is, the more powerful and accurate the result of the study. Should you accidentally miss a day, just skip it, and continue to complete the app task as normal. If possible, please try to avoid submitting multiple questionnaires a day.

Appendix F4 RESIST instruction booklet; Immediate cessation condition (excl. PACO app installation guide and FAQ section)

UCL

The REduction of Sugar In tea Study

RESIST

INSTRUCTION BOOKLET

Thank you for participating in the RESIST study.

This booklet contains all the information you will need throughout the 4 weeks of RESIST.

We suggest that you start by reading the entire booklet, and we encourage you to **print a copy** so that you can make notes on the pages. We have included a number of handy tips to help you get started and to keep you on track with your goal of reducing the amount of added sweetness in your tea.

A guide on how to install the PACO app on your phone can be found in the last section of this booklet. This app will be sending you daily notifications to complete a short daily questionnaire.

Don't worry if this is intimidating to begin with, the longer you will stick to your sugar reduction protocol, the easier it will become. Even if you forget to complete your PACO app logging task on some days, we would still like you to **complete it as often as possible** (irrespective of whether you manage to stick to your sugar reduction schedule).

If you have any questions, please contact Andrea Smith:
E-mail: andrea.smith.14@ucl.ac.uk
Telephone: 020 7679 1736

In this booklet you will find:

2

TIPS TO REDUCE & TRACK SUGAR IN TEA

- When you start to reduce sugar in tea, it is likely that for the first 3-5 days you might feel like your tea has no taste or you might not enjoy it as much. But you'll get used to it very quickly once you start to taste the 'real' aroma of tea and this can be very pleasant.
- Remove the sugar you keep close to your tea bags. Alternatively, put it away in a different cupboard to avoid automatically adding the sugar.
- If anybody offers you a cup of tea, let them know that you don't take sugar any more. If people question your new behaviour, have a response ready, e.g. "I prefer it this way", or "I am making healthy changes".
- Tell your friends and family about your intention to reduce the amount of sugar in your tea; group support will encourage you to stick it out.
- Reward yourself once a week for sticking to your schedule. Mini rewards could include:
  - Buying yourself a fancy new mug or tea to-go container
  - Buy an interesting new tea that you have always wanted to try
  - Post about your successes on Twitter, Facebook or any other social media
  - Plan a night out with your friends
  - Add £1 to a coin jar for every day you stick to your protocol – the growing collection of money will be a visual reminder of what you've accomplished and will motivate you to add to it
  - Treat yourself to a magazine

HEALTH BENEFITS OF REDUCING SUGAR INTAKE

3

TIPS TO TRACK SUGAR IN TEA

For the purpose of this study, we would like you to initially track and reduce ANY added sweetness to your beverages. This includes white sugar, brown sugar, flavoured syrups, honey, agave syrup and/or any artificial sweeteners.

When measuring one teaspoon of sugar, please ensure that it is NOT heaped.

1 teaspoon of sugar = 4 grams

= 3 - 4 grams of sugar (average)

= 5 grams of sugar (average)

4

You will be eliminating sweetness in tea immediately

To get you started, please install the smartphone app 'PACO' https://www.pacoapp.com.

Detailed installation guidelines can be found at the end of this booklet. To use the app, you will have to **use your allocated Gmail account** (e.g. 2017resist.XX@gmail.com) which will be sent to you in an e-mail.

The study will consist of 3 main phases:

(1) **Warm-up phase** (starting on Monday 6/2): Maintain your usual eating/drinking behaviours for 2 days but start using the RESIST smartphone app to track your tea liking, and the amount of sugar that you add to your tea on an average day.

(2) **Start of the intervention schedule** (Wednesday 8/2): During this 1-month period you will be given specific instructions on how to cut-back on the amount of sugar in your tea. We ask you to complete a daily questionnaire via the app during this phase.

(3) **Completion phase:** At the end of the study, you will complete a 5-minute questionnaire sent to you via e-mail. Following this, you will receive a further 5-min follow-up questionnaire a couple of weeks after the study has been completed. Once this final questionnaire has been returned, then you will receive your Amazon voucher.

- On Sunday the 5/2, you will receive an e-mail to let you know once the RESIST 'warm-up' phase is about to start.

DON'T FORGET

You will have received a set of measuring spoons that will help you to track precisely how much sugar you add to a typical cup.

- You will then be sent a further e-mail to signal the start of the RESIST schedule. From Wednesday the 8/2 onwards (Day 1), we ask you to **stop using sugar, honey or artificial sweeteners to sweeten your tea.** This might be quite challenging at first but research suggests that this immediate approach is an effective way to decrease your preference for sweetness in tea.
- The app on your phone will send you a daily reminder every evening to fill in the 4 short questions on your liking of the sweetness level of your tea for that day. Please take the time to complete this 2-minute task every day.
- At the end of the study period we will be sending you a follow-up questionnaire to ask you about your general experience of the study. Shortly afterwards you will receive a £10 Amazon voucher as a thank you for your participation.

Number of teaspoons of sugar in my tea at the start of the study ___

Appendix F5 RESIST instruction booklet; Waiting control condition (excl. PACO app installation guide and FAQ section

UCL

The REduction of Sugar In tea Study

RESIST

INSTRUCTION BOOKLET

Thank you for participating in the RESIST study.

This booklet contains all the information you will need throughout the 4 weeks of RESIST.

We suggest that you start by reading the entire booklet, and we encourage you to **print a copy** so that you can make notes on the pages. We have included a number of handy tips to help you get started and to keep you on track with your goal of reducing the amount of added sweetness in your tea.

A guide on how to install the PACO app on your phone can be found in the last section of this booklet. This app will be sending you daily notifications to complete a short daily questionnaire.

Don't worry if this is intimidating to begin with, the longer you will stick to your sugar reduction protocol, the easier it will become. Even if you forget to complete your PACO app logging task on some days, we would still like you to **complete it as often as possible** (irrespective of whether you manage to stick to your sugar reduction schedule).

If you have any questions, please contact Andrea Smith:
E-mail: andrea.smith.14@ucl.ac.uk
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- Remove the sugar you keep close to your tea bags. Alternatively, put it away in a different cupboard to avoid automatically adding the sugar.
- If anybody offers you a cup of tea, let them know that you don't take sugar any more. If people question your new behaviour, have a response ready, e.g. "I prefer it this way", or "I am making healthy changes".
- Tell your friends and family about your intention to reduce the amount of sugar in your tea; group support will encourage you to stick it out.
- Reward yourself once a week for sticking to your schedule. Mini rewards could include:
  - Buying yourself a fancy new mug or tea to-go container
  - Buy an interesting new tea that you have always wanted to try
  - Post about your successes on Twitter, Facebook or any other social media
  - Plan a night out with your friends
  - Add £1 to a coin jar for every day you stick to your protocol – the growing collection of money will be a visual reminder of what you've accomplished and will motivate you to add to it
  - Treat yourself to a magazine

HEALTH BENEFITS OF REDUCING SUGAR INTAKE

TIPS TO TRACK SUGAR IN TEA

For the purpose of this study, we would like you to initially track and reduce ANY added sweetness to your beverages. This includes white sugar, brown sugar, flavoured syrups, honey, agave syrup and/or any artificial sweeteners.

When measuring one teaspoon of sugar, please ensure that it is NOT heaped.

1 teaspoon of sugar = 4 grams

= 3 - 4 grams of sugar (average)

1 pump of flavoured syrup = 5 grams of sugar (average)

YOUR RESIST SUGAR SCHEDULE

You will be monitoring your behaviour for 4 weeks before you initiate your sweetness preference reduction protocol

To get you started, please install the smartphone app 'PACO' https://www.pacoapp.com.

Detailed installation guidelines can be found at the end of this booklet. To use the app, you will have to **use your allocated Gmail account** (e.g. 2017resist.XX@gmail.com) which will be sent to you in an e-mail.

The study will consist of 3 main phases:

(1) **Warm-up phase** (starting on Monday 6/2): Maintain your usual eating/drinking behaviours for 4 weeks but start using the RESIST smartphone app to track your tea liking, and the amount of sugar that you add to your tea on an average day.

(2) **Start of the intervention schedule** (Wednesday 8/2): During this 1-month period you will be asked to complete a daily questionnaire via the app. This will allow you to gain an accurate insight into your habitual drinking behaviours.

(3) **Completion phase:** At the end of the study, you will complete a 5-minute questionnaire sent to you via e-mail. Following this, you will receive a further guide that will enable you to implement certain habits to reduce your preference for sweetness in tea. Once this final questionnaire has been returned, then you will receive your Amazon voucher.

YOUR RESIST SUGAR SCHEDULE

- On Sunday the 5/2, you will receive an e-mail to let you know that the warm-up phase of the RESIST study has started. For the **first 4 weeks** of the study we ask you to **maintain your usual eating/drinking behaviours** and to start using the RESIST smartphone app to track your tea liking, and the amount of sugar that you add to your tea on an average day.

DON'T FORGET

You will have received a set of measuring spoons that will help you to track precisely how much sugar you add to a typical cup.

- The app will send you a short questionnaire every evening to complete 4 short questions on your liking of the sweetness level of your tea for that day. Please take the time to complete this 2-minute task every day.
- After the first month you will then be sent a further e-mail to signal the start of the active self-directed RESIST phase. From this day, you will receive a further set of instructions that will explain in detail how to complete a number of steps to reduce the liking of sweetness in your tea.
- At the end of the first 4 weeks we will be sending you a follow-up questionnaire to ask you about your general experience of the monitoring phase study. Once you have completed this questionnaire, then we will send you a £10 Amazon voucher as a thank you for your participation. You will then also receive further materials to actively reduce your liking of sweetness in tea.

Number of teaspoons of sugar in my tea at the start of the study ___

Appendix F6 Information sheet sent to participants accompanying the sugar measurement spoons

DEPARTMENT OF BEHAVIOURAL SCIENCE AND HEALTH

Thank you for participating in the REduction of Sugar in Tea STudy (RESIST).

For the purpose of the study, we are sending you a set of measuring spoons which will allow you to accurately estimate the amount of sugar added to your hot beverages.

On this spoon set you will find the following measures: 1 tablespoon (TBS), ½ TBS, 1 teaspoon (TSP), ½ TSP, ¼ TSP and 1/8 TSP.

What happens next?

- In about one week from now you will receive an electronic study guide (via e-mail) which will explain the next steps for this study.
- For now, please place your spoons in a location so that you do not lose them, and regularly check your e-mail inbox for the next RESIST update.

If you have any queries, please contact Andrea Smith: andrea.smith.14@ucl.ac.uk.

Appendix F7 Participant information sheet before start of RESIST

DEPARTMENT OF BEHAVIOURAL SCIENCE AND HEALTH

Dear Participant,

Thank you for taking part in the RESIST study.
You may have just received my previous e-mail with the invitation to complete the final questionnaire that is part of the study. Please do complete this questionnaire before you read this e-mail in more detail.

Don't forget that completing this 5-minute questionnaire will be rewarded with a £10 Amazon voucher.

Please find attached your personal RESIST instruction booklet. By now you should have received a set of measuring spoons, either during your in-person experimental objective sweetness preference assessment, or by post. RESIST is about learning how to reduce your preference for sweetness in tea. Previous research has suggested that different taste preference modification strategies may be effective in reducing the liking of sweetness in food, but this has not been scientifically established in drinks. Your participation in RESIST will contribute to a body of scientific research that aims to improve health and weight maintenance by establishing the most effective strategy to decrease the liking for sweetness in drinks.

How to complete RESIST?
The RESIST 'warm-up' phase begins on Monday the 6th of February. Please ensure that you have read your booklet by that date as it contains your tailored sugar reduction goals and instructions on how to download the smartphone application. Further necessary information to guide you through the study are all outlined in this booklet too.
We suggest that you download the app as soon as you receive this e-mail. A personal Gmail account has been created for you which will allow you to access the app. Your log-on details are:

Your personal Gmail login: 2017resist.xx@gmail.com
Your password: resist2017

You will be sent a daily reminder to complete a short questionnaire in the evening (around 9:30 pm) via this app. If possible, we would like you to complete this task every day.

Step 1 - Today
Read your booklet & install the app on your smartphone.

→ **Step 2 – 'Warm-up'**
(Monday 6/2)
Complete the 'warm up' phase to familiarize yourself with the app.

→ **Step 3 – START**
(Wednesday 8/2)
Complete the app task once a day for 4 weeks.

→ **Step 4 – FINISH**
(Friday 10/3)
Fill-in the RESIST completion questionnaire (5 min).

→ **Step 5 - Optional**
Optional experimental taste test &/or interview. Last questionnaire. Voucher distribution.

A financial incentive (£10 Amazon voucher) will be offered for participation, and these vouchers will be awarded after completion of the final questionnaire (4 weeks after intervention completion).
If you have any questions, please do not hesitate to contact Andrea Smith: andrea.smith.14@ucl.ac.uk.

Yours sincerely,

The RESIST Team
Research Department of Behavioural Science and Health
University College London
1-19 Torrington Place
London WC1E 6BT, UK

Approved by the UCL Research Ethics Committee – Project ID number 10006/001.
All data will be collected and stored in accordance with the Data Protection Act 1998.

Appendix F8 Baseline questionnaire pre-randomisation for RESIST

RESIST - Baseline

Thank you for your interest in our research.

The aim of this questionnaire is:
1) to find out about your liking for sweet foods and drinks in general, and
2) to find out which hot drinks you tend to drink, and whether or not you take sugar in these.

We will also ask you a few basic things about yourself – e.g. your age and sex. This study has been approved by the UCL Research Ethics Committee (Project ID number 10005/001). In total this survey will take about 15 minutes to complete. It is very important to us that you take the time to answer the questions correctly; your input is very valuable to us.

A financial incentive (£10 Amazon voucher) will be offered for participation, and these vouchers will be awarded after completion of the intervention.

By completing this survey, and by ticking the boxes below, you are agreeing to participate in a 4-week study about giving up sugar in your tea. You will receive a daily reminder on your smartphone to complete 4 short questions each day, which should take no longer than 3 minutes to complete.

If you have any further questions about the study, please email Andrea Smith: andrea.smith.14@ucl.ac.uk.

If you are happy to take part in this study, please click on 'Next' below. By clicking on "Next" you are agreeing and consenting that:

- You have read the notes written above and you understand what the survey involves
- You understand that you are free to withdraw from the study at any time without giving a reason

Please complete the following information:

1

RESIST - Baseline

* 1. Do you drink tea with sugar at all?

- ◯ Yes
- ◯ No

RESIST - Baseline

ABOUT YOU

* 2. What is your age?

Age

* 3. What is your gender?

- Female
- Male
- Other (specify)

* 4. Please enter your e-mail address:

* 5. Which operating system does your mobile phone device use?

- iOS (all Apple phones)
- Android
- Other (please specify)

* 6. What best describes your ethnic origin?

- White British
- White Irish
- Any other White
- Black African
- Black Caribbean
- Any other Black
- Indian
- Bangladeshi
- Pakistani
- Chinese
- Any other Asian (please specify)
- Mixed White and Black African
- Mixed White and Black Caribbean
- Mixed White and Asian
- Any other mixed
- Other (please specify)

* 7. What best describes your current relationship status?

- Single
- In a relationship
- Married
- Other
- Other (please specify)

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* 8. What is the current level of education that you are completing?

- ◯ Undergraduate Degree
- ◯ Master's degree (or equivalent)
- ◯ PhD (or equivalent)
- ◯ Other
- ◯ Other (please specify)

* 9. Please select the option which best describes your current living arrangement (main residence)

- ◯ Living at home with parents
- ◯ Living in student halls
- ◯ Living own flat/house
- ◯ Living in shared flat/house
- ◯ Living in University/College residential accommodation
- ◯ Other (please specify)

* 10. What is your term time home address?

Line 1

Line 2

Postcode

City

Don't worry, we will not abuse your details or pass them on to any third parties. All your personal data will be stored in compliance with the 1998 Data Protection Act.
We are only asking you for your home and e-mail address so that we can send you all the necessary material that you will receive as part of this research study.

5

RESIST - Baseline

ABOUT YOUR HEALTH

* 11. What is your current weight approximately? Please give this in:

Stones and pounds (e.g. 10st 5lb)

OR Pounds (e.g. 145lb)

OR Kilograms (e.g. 65.8kg)

* 12. What is your height approximately? Please give this in:

Feet and inches (e.g. 5ft 9in)

OR Inches (e.g. 68.9in)

OR Centimetres (e.g. 175cm)

* 13. How would you describe your current weight?

- Very underweight
- Underweight
- About the right weight
- Somewhat overweight
- Very overweight
- Obese

* 14. Which statement best describes you?

- I'm not bothered about my weight
- I watch my weight to keep it where it is now
- I'm trying to lose weight
- I'm trying to gain weight

6

* 15. In general, would you say your health is:

- ◯ Poor
- ◯ Fair
- ◯ Good
- ◯ Very good
- ◯ Excellent

* 16. Are you or have you ever considered yourself a smoker?

- ◯ Yes, I am currently a daily smoker
- ◯ Yes, I occasionally smoke
- ◯ I used to be a daily smoker
- ◯ I used to be an occasional smoker
- ◯ I have never smoked

* 17. Are you currently on a restrictive (weightloss) diet or have you been in the past year?

- ◯ Yes
- ◯ No
- ◯ Other (please specify)

* 18. Are you pregnant?

- ◯ Yes
- ◯ No

* 19. Have you ever been diagnosed with diabetes (type 1 or 2)?

- ◯ Yes
- ◯ No

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* 20. Briefly read the following list of drinks and tick the box which most accurately reflects how much (on average) you LIKE the specific drink (not necessarily how much you actually consume). For any drinks you don't know, or don't remember ever having tried, please select "Not applicable".

	Like a lot	Like a little	Neither like nor dislike	Dislike a little	Dislike a lot	Not applicable
Milk (dairy)	○	○	○	○	○	○
Flavoured milk (e.g. Milkshakes)	○	○	○	○	○	○
Coffee (unsweetened)	○	○	○	○	○	○
Coffee (sweetened to your liking)	○	○	○	○	○	○
Tea (unsweetened)	○	○	○	○	○	○
Tea (sweetened to your liking)	○	○	○	○	○	○
Hot Chocolate	○	○	○	○	○	○
Regular fizzy drinks (e.g. Coca Cola)	○	○	○	○	○	○
Diet fizzy drinks (e.g. Coca Cola Zero)	○	○	○	○	○	○
Energy drinks (e.g. Red Bull)	○	○	○	○	○	○
Diet Energy drinks (e.g. Diet Red Bull)	○	○	○	○	○	○
100% Fruit Juice	○	○	○	○	○	○
Juice drinks (e.g. Oasis)	○	○	○	○	○	○
Fruit Squash (added sugar)	○	○	○	○	○	○
Fruit Squash (artificially sweetened)	○	○	○	○	○	○
Fruit smoothies	○	○	○	○	○	○
Fruit & vegetable smoothies	○	○	○	○	○	○

* 21. Briefly read the following list of drinks and tick the box which most accurately reflects how frequently (on average) you CONSUME the specific drink. For any drinks you don't know, or don't remember ever having tried, please select "Not applicable".

	Never	1x month	2-3x month	1x week	2-3x week	3-4x week	5-6x week	1x day	2-3x day	4-5x day	>6x day	Not Applicable
Milk (dairy)												
Flavoured milk (e.g. Milkshakes)												
Coffee (unsweetened)												
Coffee (sweetened to your liking)												
Tea (unsweetened)												
Tea (sweetened to your liking)												
Hot Chocolate												
Regular fizzy drinks (e.g. Coca Cola)												
Diet fizzy drinks (e.g. Coca Cola Zero)												
Energy drinks (e.g. Red Bull)												
Diet Energy drinks (e.g. Diet Red Bull)												
100% Fruit Juice												
Juice drinks (e.g. Oasis)												
Fruit Squash (added sugar)												
Fruit Squash (artificially sweetened)												
Fruit smoothies												
Fruit & vegetable smoothies												

RESIST - Baseline

ABOUT YOUR TEA DRINKING

* 22. How old were you when you started drinking tea?

0 80

* 23. On average, how much sugar do you put in a cup of tea (~200ml)?

- 0 teaspoons
- 0.5 teaspoon
- 1 teaspoon
- 1.5 teaspoon
- 2 teaspoons
- 2.5 teaspoons
- 3 teaspoons
- 3.5 teaspoons
- 4 teaspoons
- 4.5 teaspoons
- 5 teaspoons
- >5 teaspoons

* 24. Do you ever add artificial sweeteners into your tea, instead of sugar?

- No
- Yes (please specify which type of sweetener and quantity per average cup)

* 25. Do you add milk or creamer into your tea?

- No
- Rarely
- Occasionally
- Yes, always about 1 tablespoon
- Yes, always about 2 tablespoons
- Yes, always about 3 tablespoons
- About 50% tea, 50% milk or creamer
- Other (please specify)

* 26. Please indicate how many cups of tea you would drink during different times of the day (on average):

	1 cup	2 cups	3 cups	4 cups	5 cups	N/A
In the morning until midday						
From midday until 6pm						
From 6pm until you go to bed						

Other (please specify)

* 27. How much do you like the sweetness level of your tea today?

1 - Dislike a lot	2	3	4	5	6	7	8	9 - Like a lot
☆	☆	☆	☆	☆	☆	☆	☆	☆

* 28. How often do you have a sweet snack (e.g. biscuit or cake) with your tea?

- Never
- Rarely (about 1x/week)
- Occasionally (2-5x/week)
- Often (1x/day)
- Nearly every time

* 29. Have you ever attempted to reduce or quit sugar in your tea?

- ◯ No, never
- ◯ Yes, I tried to reduce/quit but it didn't work
- ◯ Yes, I tried to reduce/quit once before and was successful for a while but then went back to adding (more) sugar in my tea
- ◯ Yes, I successfully reduced the sugar in my tea and have maintained this lower level of preferred sweetness
- ◯ Other (please specify)

* 30. How strongly do you agree with the following statement: I want to reduce/eliminate added sugar in my tea?

0 (Disagree) 100 (Strongly agree)

* 31. How you would like to reduce sugar in tea?
(This answer does not influence your actual intervention assignment in the study)

- ◯ Abrupt/Immediate elimination
- ◯ Gradual reduction
- ◯ No preference

RESIST - Baseline

ABOUT YOUR COFFEE DRINKING:

* 32. Do you drink coffee?

- Yes
- Rarely
- No

33. How old were you when you started drinking coffee?

0 80

34. On average, how much sugar do you put in a cup of coffee (~200ml)?

- 0 teaspoons
- 0.5 teaspoon
- 1 teaspoon
- 1.5 teaspoon
- 2 teaspoons
- 2.5 teaspoons
- 3 teaspoons
- 3.5 teaspoons
- 4 teaspoons
- 4.5 teaspoons
- 5 teaspoons
- >5 teaspoons
- I do not drink coffee

* 38. How often do you have a sweet snack (e.g. biscuit or cake) with your coffee?

- ◯ Never
- ◯ Rarely (about 1x/week)
- ◯ Occasionally (2-5x/week)
- ◯ Often (1x/day)
- ◯ Nearly every time
- ◯ I don't drink coffee

39. How much do you like the sweetness level of your coffee today?

1- Dislike a lot	2	3	4	5	6	7	8	9 - Like a lot
☆	☆	☆	☆	☆	☆	☆	☆	☆

Please select this option if you do not drink coffee

40. Have you ever attempted to reduce or quit sugar in your coffee?

- ◯ No, never
- ◯ Yes, I tried to reduce/quit but it didn't work
- ◯ Yes, I tried to reduce/quit once before and was successful for a while but then went back to adding (more) sugar in my coffee
- ◯ Yes, I successfully reduced the sugar in my coffee and have maintained this lower level of preferred sweetness
- ◯ I do not drink coffee
- ◯ Other (please specify)

41. How strongly do you agree with the following statement: I want to reduce/eliminate added sugar in my coffee? Please skip this question if you do not drink coffee.

0 (Disagree) 100 (Strongly agree)

RESIST - Baseline

ABOUT YOUR LIKING FOR SWEET FOODS

* 42. Would you say you have a "sweet tooth"?

- ◯ Not at all
- ◯ Not really
- ◯ Neither agree nor disagree
- ◯ Somewhat
- ◯ Very much

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RESIST - Baseline

OPTIONAL TASKS

We are also conducting two additional studies to enhance the accuracy of RESIST. You will receive an additional £10 for each completed task on top of the initial £10. Below are short summaries of these supplementary tasks which will be happening in parallel to the main study.

It is up to you to decide whether to take part or not in these extra activities; choosing not to take part will not disadvantage you in any way. If you do decide to take part, you are still free to withdraw at any time and without giving a reason.

* 43. Opt-in opportunity 1:

Before and at the end of the 4-week intervention period we would like to undertake a short in-person experimental objective sweetness preference assessment This would involve the sampling of different solutions and you would rate how much you like them. These sessions will be hosted in a UCL building and timings will be flexible.

Please indicate if you would be interested in taking part.

- ◯ Yes
- ◯ No

* 44. Opt-in opportunity 2:

At the end of the 4 weeks, we would like to interview you (via a phone call or in-person at UCL), to give us feedback as to whether you benefited from the intervention, whether the program was useful to you, easy to carry out, whether you would have preferred something different and how satisfied you felt with the results.

Please indicate if you would be interested in taking part.

- ◯ Yes
- ◯ No

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Appendix F9 Completion questionnaire for RESIST [T3]

RESIST - Completion questionnaire [T3]

Thank you for your completing the RESIST study protocol over the past 4-weeks!
Well done for sticking with it, you are nearly finished with this study.

The aim of this questionnaire is:
1) to collect your thoughts on the RESIST study,
2) to find out if there have been any changes in the hot drinks you tend to drink, and whether your liking for sweetness may have reduced over the past weeks.

In total this survey will take about 5-10 minutes to complete.
You will receive a shorter version of this questionnaire in 4 weeks again (on the 7th of April).
This questionnaire will be the final questionnaire.

Your financial reward (£10 Amazon voucher) will be given after completion of the questionnaire in April..

If you have any further questions about the study, please email Andrea Smith: andrea.smith.14@ucl.ac.uk.

Please click 'Next' to proceed to the questionnaire.

1

RESIST - Completion questionnaire [T3]

ABOUT YOU

* 1. First name?

* 2. Surname?

* 3. Please enter your RESIST Gmail address:

RESIST - Completion questionnaire [T3]

ABOUT YOUR CURRENT TEA DRINKING

* 4. On average, how much sugar do you put in a cup of tea (~200ml)?

- ◯ 0 teaspoons
- ◯ 0.5 teaspoon
- ◯ 1 teaspoon
- ◯ 1.5 teaspoon
- ◯ 2 teaspoons
- ◯ 2.5 teaspoons
- ◯ 3 teaspoons
- ◯ 3.5 teaspoons
- ◯ 4 teaspoons
- ◯ 4.5 teaspoons
- ◯ 5 teaspoons
- ◯ >5 teaspoons

* 5. Do you ever add artificial sweeteners into your tea, instead of sugar?

- ◯ No
- ◯ Yes (please specify which type of sweetener and quantity per average cup)

* 6. Do you add milk or creamer into your tea?

- ◯ No
- ◯ Rarely
- ◯ Occasionally
- ◯ Yes, always about 1 tablespoon
- ◯ Yes, always about 2 tablespoons
- ◯ Yes, always about 3 tablespoons
- ◯ About 50% tea, 50% milk or creamer
- ◯ Other (please specify)

* 7. Please indicate how many cups of tea you would drink during different times of the day (on average):

	1 cup	2 cups	3 cups	4 cups	5 cups	N/A
In the morning until midday	◯	◯	◯	◯	◯	◯
From midday until 6pm	◯	◯	◯	◯	◯	◯
From 6pm until you go to bed	◯	◯	◯	◯	◯	◯

Other (please specify)

* 8. How much do you like the sweetness level of your tea today?

1 - Dislike a lot	2	3	4	5	6	7	8	9 - Like a lot
☆	☆	☆	☆	☆	☆	☆	☆	☆

* 9. How often do you have a sweet snack (e.g. biscuit or cake) with your tea?

- ◯ Never
- ◯ Rarely (about 1x/week)
- ◯ Occasionally (2-5x/week)
- ◯ Often (1x/day)
- ◯ Nearly every time

RESIST - Completion questionnaire [T3]

ABOUT YOUR EXPERIENCE OF THE RESIST STUDY

* 10. Did you find it difficult to drink your tea with a reduced amount of sugar/no sugar in it?

- Not at all
- Slightly
- Somewhat
- Very much
- Extremely
- I was not instructed to reduce the sugar/sweetness levels in my tea

Other (please specify)

* 11. Did you read the RESIST intervention booklet?

- Yes
- No

Other (please specify)

* 12. Did you find the RESIST intervention booklet useful?

- Yes
- No

Other (please specify)

RESIST - Completion questionnaire [T3]

ABOUT RESIST

Thinking about RESIST, to what extent do you agree with the following:

* 16. I learned something new from RESIST

- Strongly disagree
- Disagree
- Neither agree or disagree
- Agree
- Strongly agree

Other (please specify)

* 17. The RESIST booklet is easy to understand

- Strongly disagree
- Disagree
- Neither agree or disagree
- Agree
- Strongly agree

Other (please specify)

* 18. I would recommend RESIST to a friend

- Not at all
- Slightly
- Somewhat
- Very much
- Extremely

Other (please specify)

7

* 19. I think RESIST worked to reduce my preference for sweetness in tea

- Strongly disagree
- Disagree
- Neither agree or disagree
- Agree
- Strongly agree
- I was not instructed to decrease sugar/sweetness in my tea

Other (please specify)

* 20. I increased my consumption of sweet food to make up for not having sugar in my tea

- Strongly disagree
- Disagree
- Neither agree or disagree
- Agree
- Strongly agree
- I was not instructed to decrease sugar/sweetness in my tea

Other (please specify)

* 21. I experienced cravings for sugar/sweetness once I started to reduce the amount of sugar in my tea

- Strongly disagree
- Disagree
- Neither agree or disagree
- Agree
- Strongly agree
- I was not instructed to decrease sugar/sweetness in my tea

Other (please specify)

* 22. My craving for sugar/sweetness reduced over time

- I had no cravings
- Yes, after 3 days
- Yes, after 1 week
- Yes, after 2 weeks
- Yes, after 3 weeks
- Never, I craved sweetness throughout the intervention
- I was not instructed to decrease sugar/sweetness in my tea
- Other (please specify)

* 23. Sweet foods and drinks tasted sweeter or too sweet after the study

- Strongly disagree
- Disagree
- Neither agree or disagree
- Agree
- Strongly agree
- I was not instructed to decrease sugar/sweetness in my tea

Other (please specify)

* 24. Other foods, such as fruits and vegetables, tasted sweeter after the study?

- Strongly disagree
- Disagree
- Neither agree or disagree
- Agree
- Strongly agree
- I was not instructed to decrease sugar/sweetness in my tea

Other (please specify)

* 25. I will be adding less sugar/sweetness to my tea after RESIST

- Strongly disagree
- Disagree
- Neither agree or disagree
- Agree
- Strongly agree
- I was not instructed to decrease sugar/sweetness in my tea

Other (please specify)

* 26. I will be consuming less sweet foods overall after RESIST

- Strongly disagree
- Disagree
- Neither agree or disagree
- Agree
- Strongly agree
- I was not instructed to decrease sugar/sweetness in my tea

Other (please specify)

* 27. I have benefited from taking part in RESIST

- Strongly disagree
- Disagree
- Neither agree or disagree
- Agree
- Strongly agree
- I was not instructed to decrease sugar/sweetness in my tea

Other (please specify)

* 28. I completed the PACO app task notifications on at least 10 days (out of 31 days of the study)

- ◯ Yes
- ◯ No
- ◯ Not sure

Other (please specify)

29. If not, why not?

- ◯ I was too busy
- ◯ Every day seemed too often
- ◯ I found the RESIST instructions too complicated
- ◯ I experienced technical difficulties with the app/my phone
- ◯ I lost interest

Other (please specify)

* 30. Briefly read the following list of drinks and tick the box which most accurately reflects how much (on average) you LIKE the specific drink (not necessarily how much you actually consume). For any drinks you don't know, or don't remember ever having tried, please select "Not applicable".

	Like a lot	Like a little	Neither like nor dislike	Dislike a little	Dislike a lot	Not applicable
Milk (dairy)	◯	◯	◯	◯	◯	◯
Flavoured milk (e.g. Milkshakes)	◯	◯	◯	◯	◯	◯
Coffee (unsweetened)	◯	◯	◯	◯	◯	◯
Coffee (sweetened to your liking)	◯	◯	◯	◯	◯	◯
Tea (unsweetened)	◯	◯	◯	◯	◯	◯
Tea (sweetened to your liking)	◯	◯	◯	◯	◯	◯
Hot Chocolate	◯	◯	◯	◯	◯	◯
Regular fizzy drinks (e.g. Coca Cola)	◯	◯	◯	◯	◯	◯
Diet fizzy drinks (e.g. Coca Cola Zero)	◯	◯	◯	◯	◯	◯
Energy drinks (e.g. Red Bull)	◯	◯	◯	◯	◯	◯
Diet Energy drinks (e.g. Diet Red Bull)	◯	◯	◯	◯	◯	◯
100% Fruit Juice	◯	◯	◯	◯	◯	◯
Juice drinks (e.g. Oasis)	◯	◯	◯	◯	◯	◯
Fruit Squash (added sugar)	◯	◯	◯	◯	◯	◯
Fruit Squash (artificially sweetened)	◯	◯	◯	◯	◯	◯
Fruit smoothies	◯	◯	◯	◯	◯	◯
Fruit & vegetable smoothies	◯	◯	◯	◯	◯	◯

RESIST - Completion questionnaire [T3]

* 31. Briefly read the following list of drinks and tick the box which most accurately reflects how frequently (on average) you CONSUME the specific drink. For any drinks you don't know, or don't remember ever having tried, please select "Not applicable".

	Never	1x month	2-3x month	1x week	2-3x week	3-4x week	5-6x week	1x day	2-3x day	4-5x day	>6x day	Not Applicable
Milk (dairy)	◯	◯	◯	◯	◯	◯	◯	◯	◯	◯	◯	◯
Flavoured milk (e.g. Milkshakes)	◯	◯	◯	◯	◯	◯	◯	◯	◯	◯	◯	◯
Coffee (unsweetened)	◯	◯	◯	◯	◯	◯	◯	◯	◯	◯	◯	◯
Coffee (sweetened to your liking)	◯	◯	◯	◯	◯	◯	◯	◯	◯	◯	◯	◯
Tea (unsweetened)	◯	◯	◯	◯	◯	◯	◯	◯	◯	◯	◯	◯
Tea (sweetened to your liking)	◯	◯	◯	◯	◯	◯	◯	◯	◯	◯	◯	◯
Hot Chocolate	◯	◯	◯	◯	◯	◯	◯	◯	◯	◯	◯	◯
Regular fizzy drinks (e.g. Coca Cola)	◯	◯	◯	◯	◯	◯	◯	◯	◯	◯	◯	◯
Diet fizzy drinks (e.g. Coca Cola Zero)	◯	◯	◯	◯	◯	◯	◯	◯	◯	◯	◯	◯
Energy drinks (e.g. Red Bull)	◯	◯	◯	◯	◯	◯	◯	◯	◯	◯	◯	◯
Diet Energy drinks (e.g. Diet Red Bull)	◯	◯	◯	◯	◯	◯	◯	◯	◯	◯	◯	◯
100% Fruit Juice	◯	◯	◯	◯	◯	◯	◯	◯	◯	◯	◯	◯
Juice drinks (e.g. Oasis)	◯	◯	◯	◯	◯	◯	◯	◯	◯	◯	◯	◯
Fruit Squash (added sugar)	◯	◯	◯	◯	◯	◯	◯	◯	◯	◯	◯	◯
Fruit Squash (artificially sweetened)	◯	◯	◯	◯	◯	◯	◯	◯	◯	◯	◯	◯
Fruit smoothies	◯	◯	◯	◯	◯	◯	◯	◯	◯	◯	◯	◯
Fruit & vegetable smoothies	◯	◯	◯	◯	◯	◯	◯	◯	◯	◯	◯	◯

RESIST - Completion questionnaire [T3]

32. Any other comments or feedback?

IMPORTANT NOTICE ABOUT THE OPTIONAL TASKS:

(1) EXPERIMENTAL TASTE TESTS:
We are starting our second round of experimental taste tests in the next week, from Monday the 13th of March until Friday the 17th of March. You will have received a separate invitation by e-mail to arrange your appointment. Please respond to this e-mail directly to schedule your experimental session. You will be eligible for your additional £10 (on top of the initial £10) if you attend this second round of the experimental taste test assessment.

(2) INTERVIEWS:
Previously we asked you whether you would be willing to give an in-person or telephone interview about your experience of the RESIST study. A random sub-sample of participants has been selected for this purpose. If you have been selected, then you will be contacted in the next couple of days to arrange a convenient date for your one-on-one interview which will be undertaken by a member of our research team. You will be eligible for your additional £10 (on top of the initial £10) after your interview has been completed.

Thank you for completing this questionnaire, your input is extremely important to us.

If you have any questions you can email Andrea Smith andrea.smith.14@ucl.ac.uk.

Please click 'done' to exit.

Appendix F10 Final follow-up questionnaire for RESIST [T4]

RESIST - Final questionnaire [T4]

Thank you for your completing the RESIST study protocol.
You have now been invited to complete the last questionnaire.

The aim of this questionnaire is:
1) to collect your final thoughts on the RESIST study,
2) to find out if there have been any recent or long-lasting changes in (i) the hot drinks you tend to drink and (ii) your liking for sweetness.

In total, this survey will take about 5 minutes to complete.
This questionnaire will be the final questionnaire, and once this has been completed, then you will receive your financial reward (£10 Amazon voucher).

If you have any further questions about the study, please email Andrea Smith: andrea.smith.14@ucl.ac.uk.

Please click 'Next' to proceed to the questionnaire.

1

RESIST - Final questionnaire [T4]

ABOUT YOU

* 1. First name?

* 2. Surname?

* 3. Please enter your RESIST Gmail address:

2

RESIST - Final questionnaire [T4]

ABOUT YOUR CURRENT TEA DRINKING

* 4. On average, how much sugar do you put in a cup of tea (~200ml)?

- 0 teaspoons
- 0.5 teaspoon
- 1 teaspoon
- 1.5 teaspoon
- 2 teaspoons
- 2.5 teaspoons
- 3 teaspoons
- 3.5 teaspoons
- 4 teaspoons
- 4.5 teaspoons
- 5 teaspoons
- >5 teaspoons

* 5. Do you ever add artificial sweeteners into your tea, instead of sugar?

- No
- Yes (please specify which type of sweetener and quantity per average cup)

* 6. Do you add milk or creamer into your tea?

- No
- Rarely
- Occasionally
- Yes, always about 1 tablespoon
- Yes, always about 2 tablespoons
- Yes, always about 3 tablespoons
- About 50% tea, 50% milk or creamer
- Other (please specify)

* 7. Please indicate how many cups of tea you are drinking during different times of the day (on average):

	1 cup	2 cups	3 cups	4 cups	5 cups	N/A
In the morning until midday						
From midday until 6pm						
From 6pm until you go to bed						

Other (please specify)

* 8. How much do you like the sweetness level of your tea today?

1 - Dislike a lot	2	3	4	5	6	7	8	9 - Like a lot
☆	☆	☆	☆	☆	☆	☆	☆	☆

* 9. How often do you currently have a sweet snack (e.g. biscuit or cake) with your tea?

- Never
- Rarely (about 1x/week)
- Occasionally (2-5x/week)
- Often (1x/day)
- Nearly every time

RESIST - Final questionnaire [T4]

ABOUT RESIST

Thinking about RESIST, to what extent do you agree that the following statements apply to you TODAY?

* 10. I think RESIST worked to reduce my preference for sweetness in tea

- Strongly disagree
- Disagree
- Neither agree or disagree
- Agree
- Strongly agree
- I was not instructed to decrease sugar/sweetness in my tea

Other (please specify)

* 11. I increased my consumption of sweet food to make up for not having sugar in my tea

- Strongly disagree
- Disagree
- Neither agree or disagree
- Agree
- Strongly agree
- I was not instructed to decrease sugar/sweetness in my tea

Other (please specify)

* 12. I still experience cravings for sugar/sweetness once I started to reduce the amount of sugar in my tea

- Strongly disagree
- Disagree
- Neither agree or disagree
- Agree
- Strongly agree
- I was not instructed to decrease sugar/sweetness in my tea

Other (please specify)

* 13. My craving for sugar/sweetness has reduced over time

- I had no cravings
- Yes, after 3 days
- Yes, after 1 week
- Yes, after 2 weeks
- Yes, after 3 weeks
- Never, I craved sweetness throughout the intervention
- I was not instructed to decrease sugar/sweetness in my tea
- Other (please specify)

* 14. Sweet foods and drinks tasted sweeter or too sweet after the study, and still taste sweeter today.

- Strongly disagree
- Disagree
- Neither agree or disagree
- Agree
- Strongly agree
- I was not instructed to decrease sugar/sweetness in my tea

Other (please specify)

* 15. Other foods, such as fruits and vegetables, tasted sweeter after the study, and still taste sweeter today.

- Strongly disagree
- Disagree
- Neither agree or disagree
- Agree
- Strongly agree
- I was not instructed to decrease sugar/sweetness in my tea

Other (please specify)

* 16. I still add less sugar/sweetness to my tea after RESIST

- Strongly disagree
- Disagree
- Neither agree or disagree
- Agree
- Strongly agree
- I was not instructed to decrease sugar/sweetness in my tea

Other (please specify)

* 17. I continue to consum less sweet foods overall after RESIST

- Strongly disagree
- Disagree
- Neither agree or disagree
- Agree
- Strongly agree
- I was not instructed to decrease sugar/sweetness in my tea

Other (please specify)

* 18. I have benefited from taking part in RESIST

- Strongly disagree
- Disagree
- Neither agree or disagree
- Agree
- Strongly agree
- I was not instructed to decrease sugar/sweetness in my tea

Other (please specify)

* 19. Briefly read the following list of drinks and tick the box which most accurately reflects how much (on average) you LIKE the specific drink (not necessarily how much you actually consume). For any drinks you don't know, or don't remember ever having tried, please select "Not applicable".

	Like a lot	Like a little	Neither like nor dislike	Dislike a little	Dislike a lot	Not applicable
Milk (dairy)	◯	◯	◯	◯	◯	◯
Flavoured milk (e.g. Milkshakes)	◯	◯	◯	◯	◯	◯
Coffee (unsweetened)	◯	◯	◯	◯	◯	◯
Coffee (sweetened to your liking)	◯	◯	◯	◯	◯	◯
Tea (unsweetened)	◯	◯	◯	◯	◯	◯
Tea (sweetened to your liking)	◯	◯	◯	◯	◯	◯
Hot Chocolate	◯	◯	◯	◯	◯	◯
Regular fizzy drinks (e.g. Coca Cola)	◯	◯	◯	◯	◯	◯
Diet fizzy drinks (e.g. Coca Cola Zero)	◯	◯	◯	◯	◯	◯
Energy drinks (e.g. Red Bull)	◯	◯	◯	◯	◯	◯
Diet Energy drinks (e.g. Diet Red Bull)	◯	◯	◯	◯	◯	◯
100% Fruit Juice	◯	◯	◯	◯	◯	◯
Juice drinks (e.g. Oasis)	◯	◯	◯	◯	◯	◯
Fruit Squash (added sugar)	◯	◯	◯	◯	◯	◯
Fruit Squash (artificially sweetened)	◯	◯	◯	◯	◯	◯
Fruit smoothies	◯	◯	◯	◯	◯	◯
Fruit & vegetable smoothies	◯	◯	◯	◯	◯	◯

* 20. Briefly read the following list of drinks and tick the box which most accurately reflects how frequently (on average) you CONSUME the specific drink. For any drinks you don't know, or don't remember ever having tried, please select "Not applicable".

	Never	1x month	2-3x month	1x week	2-3x week	3-4x week	5-6x week	1x day	2-3x day	4-5x day	>6x day	Not Applicable
Milk (dairy)	◯	◯	◯	◯	◯	◯	◯	◯	◯	◯	◯	◯
Flavoured milk (e.g. Milkshakes)	◯	◯	◯	◯	◯	◯	◯	◯	◯	◯	◯	◯
Coffee (unsweetened)	◯	◯	◯	◯	◯	◯	◯	◯	◯	◯	◯	◯
Coffee (sweetened to your liking)	◯	◯	◯	◯	◯	◯	◯	◯	◯	◯	◯	◯
Tea (unsweetened)	◯	◯	◯	◯	◯	◯	◯	◯	◯	◯	◯	◯
Tea (sweetened to your liking)	◯	◯	◯	◯	◯	◯	◯	◯	◯	◯	◯	◯
Hot Chocolate	◯	◯	◯	◯	◯	◯	◯	◯	◯	◯	◯	◯
Regular fizzy drinks (e.g. Coca Cola)	◯	◯	◯	◯	◯	◯	◯	◯	◯	◯	◯	◯
Diet fizzy drinks (e.g. Coca Cola Zero)	◯	◯	◯	◯	◯	◯	◯	◯	◯	◯	◯	◯
Energy drinks (e.g. Red Bull)	◯	◯	◯	◯	◯	◯	◯	◯	◯	◯	◯	◯
Diet Energy drinks (e.g. Diet Red Bull)	◯	◯	◯	◯	◯	◯	◯	◯	◯	◯	◯	◯
100% Fruit Juice	◯	◯	◯	◯	◯	◯	◯	◯	◯	◯	◯	◯
Juice drinks (e.g. Oasis)	◯	◯	◯	◯	◯	◯	◯	◯	◯	◯	◯	◯
Fruit Squash (added sugar)	◯	◯	◯	◯	◯	◯	◯	◯	◯	◯	◯	◯
Fruit Squash (artificially sweetened)	◯	◯	◯	◯	◯	◯	◯	◯	◯	◯	◯	◯
Fruit smoothies	◯	◯	◯	◯	◯	◯	◯	◯	◯	◯	◯	◯
Fruit & vegetable smoothies	◯	◯	◯	◯	◯	◯	◯	◯	◯	◯	◯	◯

21. Any other comments or feedback?

Thank you for completing this questionnaire, your input is extremely important to us.

If you have any questions you can email Andrea Smith andrea.smith.14@ucl.ac.uk.

Please click 'done' to exit.

Appendix F11 Tracking grid used to measure psychophysical sweetness preference in RESIST

Participant Name [ID#]: ______________________

Date of experiment: ____________________

Baseline or Follow-up?

Start time: ______ End time: ________

Series 1 Pair the preferred concentration with the lower adjacent concentration + always present the weaker solution of the pair first					
A (3% w/v)	**B** (6% w/v)	**C** (12% w/v)	**D** (24% w/v)	**E** (36% w/v)	**Notes**
	X		X		

Start time: ______ End time: ________

Series 2 Pair the preferred concentration from the first comparison with the higher adjacent concentration + always present the stronger solution of the pair first					
A (3% w/v)	**B** (6% w/v)	**C** (12% w/v)	**D** (24% w/v)	**E** (36% w/v)	**Notes**
	X		X		

Appendix F12 Semi-structured interview guide for participants of RESIST

DEPARTMENT OF EPIDEMIOLOGY & PUBLIC HEALTH

UCL

Pilot study of an intervention to test the feasibility and effectiveness of sweetness preference reduction in relation to hot drinks: The REduction of Sugar In tea STudy (RESIST): Follow-up Interview

Topic guide

This topic guide is intended to ensure key aspects are covered during the interview. However, a respondent-sensitive approach will be taken, allowing deviation from the order of the questions and raising additional issues if desired.

Introduction

Introduce researcher. Introduce digital tape recorder/mention recording on Skype and ask permission to record the conversation. Assure confidentiality and that names will not be used when this is written up

Purpose of the interview: 'To explore the participant's experience of the REduction of Sugar In tea STudy (RESIST).

Interviews methods:

- In-person
- By phone
- By Skype (?)

Pilot interviews: To be scheduled after the 1st of March

Keep in mind that consent should be granted before you start recording the interview.

(A) Motivation and reason to accept the Invitation to take part in the study

1) What was the main reason you decided to take part in this study?

- Probe:
  - to improve lifestyle, energy, fatigue?
  - to feel better about self?
  - to be healthier?
  - to lose weight?

2) Did anything initially put you off?

- Probe: anything in the email information sheet or the way you were approached?
- Time constraints? Work load?

(B) RESIST intervention content

3) Upon receiving the first e-mails and the intervention booklet, did you understand what you were expected to do for the RESIST study?

4) Tell me your views about the intervention in general? Which condition did you get randomised to?

- Probe: Immediate cessation, gradual reduction?
- Did this approach align with your initial preference?
- Do you think the other approach would have been easier/more effective/more realistic for you?

5) Do you think the other intervention condition would have been easier, more appropriate, more effective for you?

- Probe: Why? Why not?

6) Did you find the sugar reduction tips in the RESIST booklet useful?

- Probe: How credible/trustworthy/reliable did you think the tips were when you received them?

7) Did you try all the tips or just a few?

- Probe: Which ones?

8) Did the intervention booklet help you to develop your own personal strategies to stick your RESIST sugar reduction protocol?

- Probe: Which ones?

2

(F) Improvements

20) Would you have reduced/stopped taking sugar in your tea anyway, even if you hadn't taken part in the study?

- Probe: New year's resolutions?

21) Do you feel as though you have benefitted from taking part in this study? How?

- Prompt: Do you think you have made changes that have improved your lifestyle?

22) What would you change about the study?

- Probe: would you like to add anything extra, such as personal contact, more information on diet, recipes?

23) Do you think you will continue with the changes you have made?

- Probe: Did tracking of your tea/sugar intake increase awareness?
- Does this increased awareness influence your future intentions about behaviour maintenance?

24) Is there anything else you would like to say about the study?

Concluding comments

Thank participant for their time on the study and during the interview
Thanks them again, and state that if they have any further comments/questions to contact Andrea Smith/Sonam Verma at any time.

Appendix F13a Perceived benefits and approval of the RESIST protocol of the active intervention groups

	Completion				4-week follow-up			
Agreement[1]	**GR** (n=20)	**IC** (n=23)	**p**[2]	**d**[3]	**GR** (n=18)	**IC** (n=23)	**p**[2]	**d**[3]
Learned something new from participating in RESIST [mean (SD)]	3.75 (.79)	3.61 (.78)	.559	.178				
RESIST booklet is easy to understand [mean (SD)]	4.25 (.91)	4.13 (.55)	.599	.159				
Recommend RESIST to a friend [mean (SD)]	3.80 (.77)	3.39 (.66)	.067	.571				
RESIST reduced preference for sweetness in tea [mean (SD)]	4.15 (.75)	3.74 (1.1)	.059	.436	4.61 (.61)	4.00 (.95)	**.023**	.764
Increased consumption of sweet food to compensate for not having sugar in tea [mean (SD)]	1.95 (.99)	2.57 (1.2)	.072	.564	2.44 (1.2)	2.30 (1.0)	.688	.127
Sweet F&D taste (too) sweet after RESIST [mean (SD)]	3.35 (1.3)	3.26 (.96)	.799	.078	3.44 (1.1)	3.57 (.95)	.714	.126
Fruit & vegetables tasted sweeter after RESIST [mean (SD)]	2.85 (1.1)	2.65 (.89)	.515	.199	3.06 (.99)	2.70 (1.1)	.287	.344
Adding less sugar/sweetness to my tea after RESIST [mean (SD)]	4.45 (.61)	4.00 (1.0)	.087	.543	4.28 (1.1)	4.39 (.84)	.706	.112
Consume less sweet foods after RESIST [mean (SD)]	3.10 (.85)	3.39 (.99)	.310	.314	3.17 (1.3)	3.78 (1.2)	.124	.488
Benefited from RESIST [mean (SD)]	4.35 (.49)	4.04 (.71)	.111	.508	4.39 (.61)	4.26 (.62)	.512	.211
Craving sweetness during RESIST [mean (SD)]	2.90 (1.3)	3.04 (1.1)	.697	.116	2.89 (1.3)	2.65 (1.0)	.515	.216

Significant findings are bolded.

[1] Agreement reported on a 5-point Likert scale from ‘Strongly disagree’ to ‘Strongly agree’; higher scores were indicative of higher agreement. Participants that selected ‘I was not instructed to reduce the sugar’ in my tea had their scores recoded to ‘neither agree/disagree’

[2] Unpaired samples t-test for comparison of continuous variables only measured at T3

[3] Cohen’s d is a measure effect size, and are interpreted as follows: ‘small’=0.2 - 0.3; ‘medium’~0.5; ‘large’ >0.8

Abbreviations: F&D= Food and drink

Appendix F13b Comparison of perceived benefits and approval of the RESIST protocol between the active intervention groups (GR and IC combined) and the WC group

Agreement[1]	Completion				4-week follow-up			
	Active (n=43)	**WC** (n=20)	**p[2,3]**	**g[3]**	**Active** (n=41)	**WC** (n=20)	**p[2,3]**	**g[3]**
Learned something new from participating in RESIST [mean (SD)]	3.67 (.78)	3.35 (.75)	.124	.415				
RESIST booklet is easy to understand [mean (SD)]	4.19 (.73)	3.95 (.83)	.257	.315				
Recommend RESIST to a friend [mean (SD)]	3.58 (.73)	3.00 (.73)	.723	.794				
RESIST reduced preference for sweetness in tea [mean (SD)]	3.86 (.94)	3.20 (.83)	**.009**	.728	4.27 (.87)	3.25 (.72)	**<.001**	1.24
Increased consumption of sweet food to compensate for not having sugar in tea [mean (SD)]	2.28 (1.1)	2.35 (.67)	.794	.071	2.37 (1.1)	2.60 (.68)	.383	.234
Sweet F&D taste (too) sweet after RESIST [mean (SD)]	3.30 (1.1)	3.20 (.52)	.700	.104	3.51 (1.0)	3.00 (.73)	**.050**	.553
Fruit & vegetables tasted sweeter after RESIST [mean (SD)]	2.74 (.98)	3.15 (.49)	.085	.478	2.85 (1.1)	2.85 (.49)	.988	0
Adding less sugar/sweetness to my tea after RESIST [mean (SD)]	4.21 (.86)	3.35 (.67)	**<.001**	1.12	4.34 (.94)	3.40 (.82)	**<.001**	1.26
Consume less sweet foods after RESIST [mean (SD)]	3.26 (.93)	3.15 (.67)	.650	.128	3.51 (1.3)	3.10 (.72)	.182	.358
Benefited from RESIST [mean (SD)]	4.19 (.63)	4.95 (1.2)	**<.001**	.895	4.32 (.61)	4.90 (1.4)	**.025**	.627
Craving sweetness during RESIST [mean (SD)]	2.98 (1.2)	2.95 (.60)	.925	.029	2.75 (1.1)	2.80 (.62)	.872	.051

Significant findings are bolded.

[1] Agreement reported on a 5-point Likert scale from ‘Strongly disagree’ to ‘Strongly agree’; higher scores were indicative of higher agreement. Participants that selected ‘I was not instructed to reduce the sugar’ in my tea had their scores recoded to ‘neither agree/disagree’

[2] Unpaired samples t-test for comparison of continuous variables only measured at T3

[3] Hedges' g is a measure of effect size (weighted per relative size of each group) taking into account unequal sample sizes). Values are interpreted as follows: ‘small’=0.2 - 0.3; ‘medium’~0.5; ‘large’ >0.8

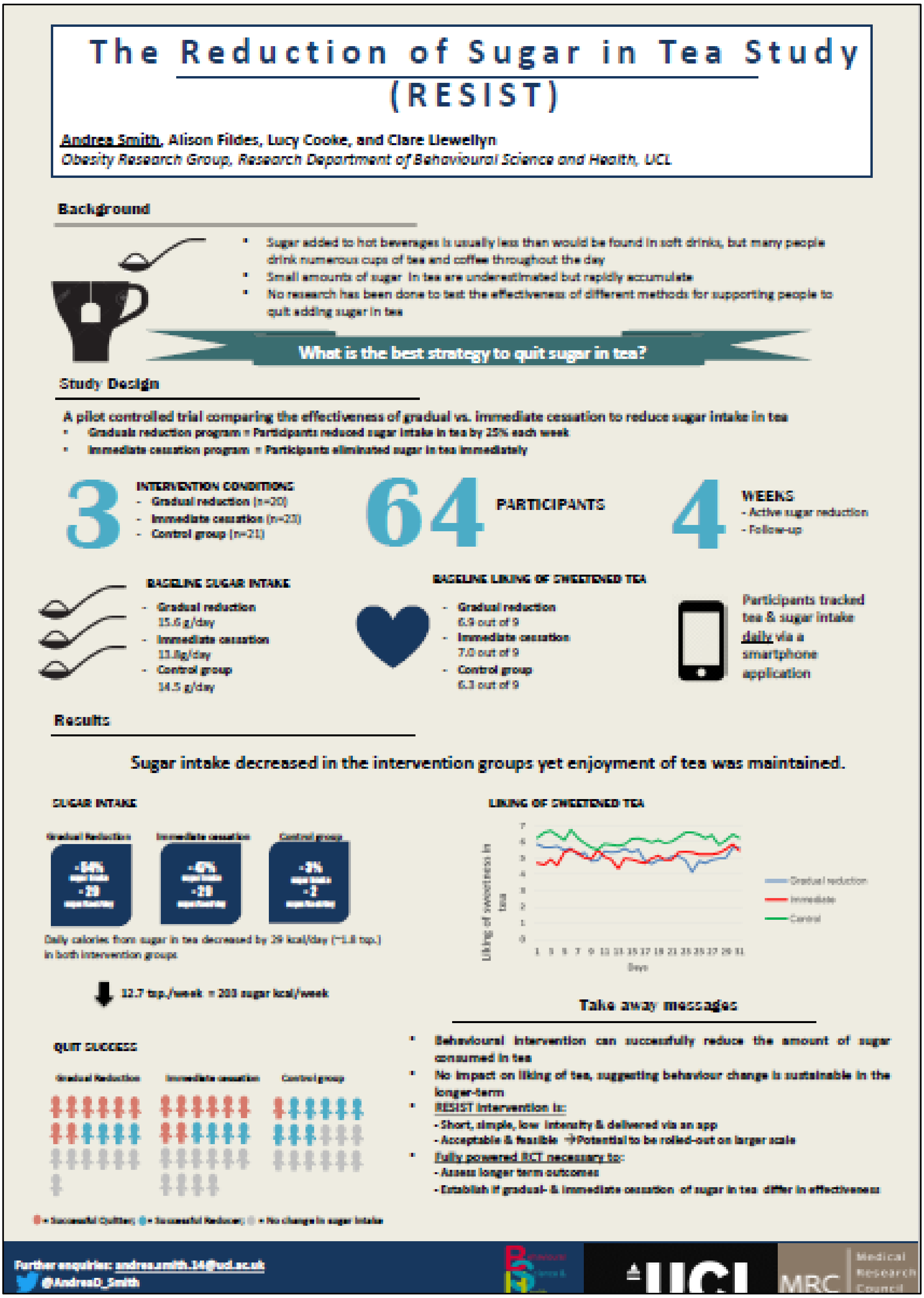
Abbreviations: F&D= Food and drink

Appendix F14 Barriers to adherence to RESIST (T4)

	Condition				
Ease[1]	**GR (n=20)**	**IC (n=23)**	**t-statistic**	**p-value**[2]	**Cohens' d**[3]
Completion of RESIST overall [mean (SD)]	1.90 (1.0)	1.87 (.69)	.116	.909	.035
Adherence to RESIST sugar reduction protocol [mean (SD)]	2.35 (1.0)	2.43 (1.1)	-.261	.795	.076
Drinking tea with reduced/no sugar [mean (SD)]	2.55 (.95)	2.43 (.95)	.399	.692	.126
Completion of daily app task [mean (SD)]	1.90 (1.0)	2.74 (.92)	-2.842	**.007**	.874
If you found it difficult to regularly complete the daily app task, why? [n (%)]					
Too busy	1 (5%)	*1* (4.3%)			
Tech difficulties with my phone/the app	5 (25%)	*2* (8.7%)			

Significant findings are bolded.
[1] Ease reported on a 5-point Likert scale, ranging from 'Very easy', 'Easy', Neither easy/difficult', 'Difficult', to 'Very difficult'; higher scores indicative of greater difficulty.
[2] Unpaired t-test for comparison of continuous variables
[3] Cohen's d is a measure effect size; Values for Cohen's d are interpreted as follows: 'small'~0.2 - 0.3; 'medium'~0.5; 'large' >0.8

Appendix F15 The Reduction of Sugar in Tea Study (RESIST)



Appendix G.

Publications

Appendix G1 Publicly available food preference questionnaire and scoring sheet for adolescents and adults

Food preference questionnaire for adolescents and adults

The food preference questionnaire requires participants to rate their liking of 62 individual foods on a 5-point Likert scale, ranging from "not at all" to "a lot". Participants are instructed to select 'not applicable' if they are not familiar with, or have no memory of having tried a food item.

Two additional questions about important food allergies or dietary requirements which may influence habitual intake and liking of certain foods are included (Q1 and Q2):

- Q1 asks whether subjects follow a pescetarian, vegetarian, or vegan dietary regimen, and
- Q2 establishes the presence of food allergies via a self-completed food allergies checklist of the 10 most frequent food allergens.

The food preference ratings can be grouped into six internally reliable categories: vegetables, fruits, meat/fish, dairy, snacks and starches.

Scoring instructions are included at the end of the questionnaire (page 5).

Contact details for further enquiries:

Andrea Smith
E-mail: andrea.smith.14@ucl.ac.uk
Telephone (UK): +44 (0) 20 7679 1736

1

1. Do you identify as any of the following?
☐Vegan
☐Vegetarian
☐Pescetarian (no meat, but eat fish and/or shellfish)
☐None of the above

2. Are you allergic to any of the following food items? (please select all that apply)
☐Peanuts
☐Tree nuts
☐Sesame
☐Dairy
☐Shellfish
☐Fish
☐Egg
☐Wheat / Gluten
☐Soya
☐Celery
☐Mustard
☐Other (please specify):

3. Please read the following list of food items and tick the box which most accurately reflects how much (on average) you like the specific item (not necessarily how much you actually consume). For any foods you don't know, or don't remember ever having tried, please select "Not applicable".

Food item	Dislike a lot	Dislike a little	Neither like nor dislike	Like a little	Like a lot	Not applicable
Beef	☐	☐	☐	☐	☐	☐
Beef burgers	☐	☐	☐	☐	☐	☐
Lamb	☐	☐	☐	☐	☐	☐
Chicken	☐	☐	☐	☐	☐	☐
Bacon	☐	☐	☐	☐	☐	☐
Ham	☐	☐	☐	☐	☐	☐
Sausages	☐	☐	☐	☐	☐	☐
White fish (e.g. cod, haddock)	☐	☐	☐	☐	☐	☐
Oily fish (e.g. mackerel, kippers)	☐	☐	☐	☐	☐	☐
Smoked salmon	☐	☐	☐	☐	☐	☐
Tinned Tuna	☐	☐	☐	☐	☐	☐
Eggs (boiled, scrambled or fried)	☐	☐	☐	☐	☐	☐
Baked beans	☐	☐	☐	☐	☐	☐
Bread or Bread rolls	☐	☐	☐	☐	☐	☐

Food item	Dislike a lot	Dislike a little	Neither like nor dislike	Like a little	Like a lot	Not applicable
Bran cereal (e.g. All Bran, Bran Flakes)	☐	☐	☐	☐	☐	☐
Porridge	☐	☐	☐	☐	☐	☐
Plain boiled rice	☐	☐	☐	☐	☐	☐
Sugared cereal (e.g. Frosties, Sugar Puffs)	☐	☐	☐	☐	☐	☐
Hummus	☐	☐	☐	☐	☐	☐
Wheat cereal (e.g. Weetabix, Shredded Wheat)	☐	☐	☐	☐	☐	☐
Potatoes (boiled or mashed)	☐	☐	☐	☐	☐	☐
Chips	☐	☐	☐	☐	☐	☐
Rice or corn cereal (e.g. Corn Flakes, Rice Krispies)	☐	☐	☐	☐	☐	☐
Soft cheese (e.g. Camembert, Brie)	☐	☐	☐	☐	☐	☐
Hard cheese (e.g. cheddar)	☐	☐	☐	☐	☐	☐
Cottage Cheese	☐	☐	☐	☐	☐	☐
Plain, low-fat yoghurt	☐	☐	☐	☐	☐	☐
Oranges	☐	☐	☐	☐	☐	☐
Grapes	☐	☐	☐	☐	☐	☐
Apples	☐	☐	☐	☐	☐	☐
Melon	☐	☐	☐	☐	☐	☐
Peaches	☐	☐	☐	☐	☐	☐
Apricots	☐	☐	☐	☐	☐	☐
Strawberries	☐	☐	☐	☐	☐	☐
Avocadoes	☐	☐	☐	☐	☐	☐
Spinach	☐	☐	☐	☐	☐	☐
Carrots	☐	☐	☐	☐	☐	☐
Green beans	☐	☐	☐	☐	☐	☐
Cucumber	☐	☐	☐	☐	☐	☐
Celery	☐	☐	☐	☐	☐	☐
Mushrooms	☐	☐	☐	☐	☐	☐
Parsnips	☐	☐	☐	☐	☐	☐
Peas	☐	☐	☐	☐	☐	☐
Sweetcorn	☐	☐	☐	☐	☐	☐
Broccoli	☐	☐	☐	☐	☐	☐
Salad leaves (e.g. lettuce)	☐	☐	☐	☐	☐	☐

Scoring of the food preference questionnaire for adults

Responses are scored 1-5, with a higher score indicative of greater liking of a food. "Not applicable" is coded as missing.

Dislike a lot = 1

Dislike a little = 2

Neither like nor dislike = 3

Like a little = 4

Like a lot = 5

Food preference scale scores for the six food categories are obtained by summing the single food preference item scores within each food category and dividing this sum by the number of items.

Food preference category	Number of items included in mean score (n)	Individual food items
Vegetables	18	Spinach, Carrots, Green beans, Cucumber, Celery, Mushrooms, Brussels sprouts, Parsnips, Peas, Sweet corn, Broccoli, Salad, Red peppers, Raw tomatoes, Avocados, Potatoes, Baked beans, Beetroot
Fruit	7	Oranges, Grapes, Apples, Melon, Peaches, Apricots, Strawberries
Meat/Fish	12	Beef, Beef burgers, Lamb, Chicken, Bacon, Ham, Sausages, White fish, Canned tuna, Oily fish, Smoked salmon, Hummus
Dairy	10	Soft cheese, Hard cheese, Eggs, Butter, Cream, Yogurt, Cottage cheese, Butter-like spread, Mayonnaise, Custard
Snacks	9	Chips, Plain biscuits, Chocolate biscuits, Cake, Ice cream, Chocolate, Crisps, Gummy sweets, Sugared cereal
Starches	6	Bread, Bran cereal, Porridge, Rice, Wheat cereal, Rice or corn cereal

Appendix G2 Published paper on the aetiology of food preferences (Chapter 5)

Genetic and environmental influences on food preferences in adolescence[1,2]

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ABSTRACT

Background: Food preferences vary substantially among adults and children. Twin studies have established that genes and aspects of the shared family environment both play important roles in shaping children's food preferences. The transition from childhood to adulthood is characterized by large gains in independence, but the relative influences of genes and the environment on food preferences in late adolescence are unknown.

Objective: The aim of this study was to quantify the contribution of genetic and environmental influences on food preferences in older adolescents.

Design: Participants were 2865 twins aged 18–19 y from the TEDS (Twins Early Development Study), a large population-based cohort of British twins born during 1994–1996. Food preferences were measured by using a self-report questionnaire of 62 individual foods. Food items were categorized into 6 food groups (fruit, vegetables, meat or fish, dairy, starch foods, and snacks) by using factor analysis. Maximum likelihood structural equation modeling established genetic and environmental contributions to variations in preferences for each food group.

Results: Genetic factors influenced a significant and substantial proportion of the variation in preference scores of all 6 food groups: vegetables (0.54; 95% CI: 0.47, 0.59), fruit (0.49; 95% CI: 0.43, 0.55), starchy foods (0.32; 95% CI: 0.24, 0.39), meat or fish (0.44; 95% CI: 0.38, 0.51), dairy (0.44; 95% CI: 0.37, 0.50), and snacks (0.43; 95% CI: 0.36, 0.49). Aspects of the environment that are not shared by 2 twins in a family explained all of the remaining variance in food preferences.

Conclusions: Food preferences had a moderate genetic basis in late adolescence, in keeping with findings in children. However, by this older age, the influence of the shared family environment had disappeared, and only aspects of the environment unique to each individual twin influenced food preferences. This finding suggests that shared environmental experiences that influence food preferences in childhood may not have effects that persist into adulthood. *Am J Clin Nutr* 2016;104:446–53.

Keywords: food preferences, behavioral genetics, heritability, twin study, cohort study

INTRODUCTION

A healthy and balanced diet is central to optimal health in both the short and long term. Food preferences are important drivers of actual food choice, determining micro- and macronutrient intakes (1–3). Poor dietary quality increases the risk of nutrition-related chronic disease, obesity (4, 5), and associated comorbidities such as type 2 diabetes (6). Understanding the etiology of food preferences therefore has important implications for policy makers and clinicians.

Twin studies have established the relative importance of genetic compared with environmental influences on food preferences in adults and children (7). A recent study in 3-y-old British children suggested moderate heritability for liking of vegetables (0.54), fruit (0.53), protein foods (0.48), snacks (0.29), starches (0.32), and dairy foods (0.27) (8). Likewise, an earlier study in 4-y-old British twins (9) found that liking of fruit (0.51), vegetables (0.37), protein foods (0.78), and dessert-type foods (0.20) all had some genetic basis, albeit with varying heritability estimates as expected from the smaller sample size. Importantly, in both studies, it was the effect of the environment shared by 2 twins in a family (the "shared environment"; e.g., being raised in the same household) that influenced food preferences, with minimal contribution from environmental influences that are unique to each child (the "nonshared environment"). This makes sense given the importance of the home family environment (e.g., food availability) for the eating behavior of preschool children (10), because the family setting is the primary environment within which a child develops his or her behaviors (11).

Studies in adult twins have also shown that food preferences tend to have a moderate genetic basis; however, the unique environment is the most important influence on adult food intake and choice (12–14), with little evidence of a meaningful influence by the shared environment (12). This indicates that shared

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[2] Supplemental Tables 1–4 and Supplemental Figure 1 are available from the "Online Supporting Material" link in the online posting of the article and from the same link in the online table of contents at http://ajcn.nutrition.org.

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environmental factors that play a role in shaping the development of food preferences in childhood are less important in adulthood, but it is unclear at what stage the influence of the shared environment declines. To our knowledge, there are no existing studies of the relative influence of genes and shared and unique environmental factors on the food preferences of older adolescents. This is an important developmental transition into adulthood that is characterized by gains in independence; at the same time, the family remains an important but diminishing source of influence as adulthood approaches.

In this study we investigate the relative magnitude of genetic, shared, and unique environmental influences on food preferences in a large sample of older adolescents (18–19 y of age). We hypothesized that food preferences would have a moderate genetic basis, in keeping with studies in both children and adults, and be influenced by both the shared and unique environment.

METHODS

Sample

Study participants were twins from the Twin Early Development Study (TEDS),[6] a birth cohort of 16,810 families with twins born in England and Wales during 1994–1996. TEDS was previously shown to be reasonably representative of the general population (15). For the current study, twins were from a subsample of twin pairs born between September 1995 and August 1996. Requests to complete the online food preference questionnaires were sent out to the entire subsample (3166 pairs; n = 6332 individuals) by letter and e-mail. Subjects were offered a £10 voucher to complete the survey, resulting in 3155 individual twins who consented to participate. Data from twins with serious medical or perinatal problems or with unknown sex or zygosity were excluded (n = 290). Of these, 52 (17.9%) were monozygotic, 156 (58.6%) were dizygotic, and 82 (21.8%) were of unknown zygosity. This breakdown is representative of typical monozygotic/dizygotic proportions observed in twin populations. Importantly, health status (a factor that conceivably influences food preferences; χ^2 = 5.918, P = 0.15), food restrictions (χ^2 = 0.26, P = 0.87), or BMI (t = 0.45, P = 0.65) did not differ by zygosity between the excluded individuals. The final sample consisted of 2865 individuals, representing 1010 complete monozygotic pairs, 909 dizygotic same-sex, and 946 dizygotic opposite-sex pairs. In addition, data were included from 379 unpaired individuals, with 90 from monozygotic, 107 from dizygotic same-sex, and 182 from dizygotic opposite-sex pairs. The procedures followed were in accordance with King's College London ethical standards on human experimentation, and approval was obtained from the relevant committee on human subjects.

Measures

Sociodemographic measures and zygosity

Date of birth, sex, birth complications, and socioeconomic information were collected in the baseline questionnaire. BMI was calculated from self-reported weight and height squared (kg/m^2). Zygosity had previously been collected by using a parental report questionnaire completed in early childhood. DNA analysis has shown the questionnaire to be >95% accurate (16); uncertain zygosity was determined from DNA.

Food preferences

Food preferences were measured via a self-report questionnaire that asked participants to rate their liking of 69 individual foods on a 5-point Likert scale, ranging from "not at all" to "a lot"; a higher score was indicative of greater liking of a food. Participants were instructed to select the "not applicable" option for foods that they had never tried. The food preference questionnaire was based on a previous questionnaire that was used to establish genetic and environmental influences on food preferences of 4-y-old children (17) to allow comparison between estimates derived from this and the previous study. For this study, revisions of the original questionnaire included the elimination of outdated food items (e.g., blancmange), the omission of composite dishes (e.g., pizza), and the addition of more contemporary foods commonly consumed by older adolescents and young adults (e.g., hummus). Principal components analysis in SPSS version 22 produced food-group factors comparable to the original food groupings (8, 9, 18). Because food preference factors are expected to correlate, an oblique rotation method was chosen (Direct oblimin).

A previous test-retest was undertaken in a sample of the twins' siblings to assess the reliability of the food preference questionnaire over a 2-wk period. Siblings (n = 205) were invited to complete the online questionnaire, with n = 94 participants completing both waves of data collection. Mean food preference score test-retest coefficients ranged from 0.61 to 0.95, which showed the questionnaire to be reasonably stable. Internal reliability (indexed by using Cronbach's α) was reasonable for the following food groups: vegetables (spinach, carrots, green beans, etc.; α = 0.89; 18 items), fruit (oranges, grapes, apples, etc.; α = 0.84; 7 items), meat or fish (beef, lamb, chicken, etc.; α = 0.81; 12 items), dairy (hard cheese, cream, yogurt, etc.; α = 0.77; 10 items), snacks (chips, cake, chocolate, etc.; α = 0.80; 9 items), and starch (bread, porridge, rice, etc.; α = 0.68; 6 items).

Food restrictions

Twins were asked whether they follow a pescatarian, vegetarian, or vegan dietary regimen. In addition, food allergy information was ascertained by using a self-completed food allergies checklist.

Statistical analysis

Twin studies are able to provide estimates of genetic influence on traits, but importantly, they are also able to separate out environmental influences into the following: *1*) those that are completely shared between 2 twins in a pair and contribute to twin similarity over and above genetics (e.g., living in the same household) and *2*) influences that are unique to each individual twin (i.e., unshared between 2 twins in a pair) and contribute to differences between twins (e.g., having different friends). Monozygotic twins are 100% genetically correlated, whereas dizygotic twins are ~50% similar genetically; however, both

[6] Abbreviations used: AIC, Akaike information criterion; ICC, intraclass correlation; MLSEM, maximum likelihood structural equation modeling; *TAS2R*, Taste 2 receptor gene family; TEDS, Twin Early Development Study.

types of twins share their environments to a similar extent. This means that resemblance between monozygotic and dizygotic twins for an observable trait (e.g., food preferences) can be compared to provide an estimate of genetic influence (indexed by the effect size indicator called "heritability," which describes the proportion of total variance that can be attributed to inherited DNA differences) and shared and unique environmental effects. Greater similarity between monozygotic twins than between dizygotic twins indicates a genetic contribution to trait variation, because researchers assume that the only difference between monozygotic and dizygotic twins is that monozygotic twins are twice as similar genetically, and their environments are shared equally (19). The extent to which monozygotic twins are different from one another provides a direct estimate of the unique environment, the only source of monozygotic pair difference (because their similarity reflects both shared genes and shared environments) other than error of measurement. Each component of variance ranges from 0% to 100%, indicating the proportion of total variation (individual differences) attributable to variation in each of genetic, shared, and unique environmental influences.

Two approaches were used to quantify the relative influence of genetic, shared, and unique environmental influences on food preference variation. Initially, food item and food category intraclass correlations (ICCs) were calculated for both monozygotic and dizygotic pairs, which indicate the pattern of the relative importance of genetic, shared, and unique environmental influences on variations in food preference scores. Maximum likelihood structural equation modeling (MLSEM) was used to derive more precise estimates of the 3 sources of variation (with 95% CIs), as well as to provide goodness-of-fit statistics. Additive genetic factors are denoted by "A," shared environmental factors by "C," and unique environmental influences by "E" (which also includes measurement error). MLSEM estimates A, C, and E based on the expected structure of the variance-covariance matrices for monozygotic and dizygotic twins, on the basis of the key assumptions of the twin design: for example, monozygotic covariation reflects the fact that the twins share all of their genes and all of their shared environments, rMZ = 1A + 1C; on the other hand, dizygotic covariation reflects the fact that the twins share half of their genes but all of their shared environments, rDZ = 0.5A +1C, where rMZ and rDZ indicate ICCs for monozygotic and dizygotic twin pairs, respectively. In an independent model, the presence of nonadditive genetic effects, denoted by "D," can be investigated. Shared environment factors and nonadditive genetic effects cannot be estimated at the same time; therefore, A, D, and E factors need to be fitted to the data in a separate model.

Initially, food preference scores were residualized for age and sex effects. This is a standard procedure in twin modeling because all twins share their age exactly (and sex for same-sex twins), and these factors can therefore inflate the shared environment effect (20). First, a saturated model was fitted, which applies no constraints to the data and simply estimates means, covariances, and variances for monozygotic and dizygotic twins. Then, a full ACE model was fitted and compared with the saturated model for goodness-of-fit, as indicated by the likelihood ratio test and the Akaike information criterion (AIC). The likelihood ratio test is a procedure used to select the best-fitting model among hierarchical nested models. The likelihood ratio statistic approximately follows a χ^2 distribution, and any addition of more variables to a model increases the likelihood score. Comparing the likelihood scores of multiple models allows for objective selection of the significantly superior model fit. Calculation of the AIC statistic penalizes for the addition of additional variables and thereby favors the simplest, most parsimonious model for the observed data. Submodels consecutively dropping the A and C variables (E is never dropped from the model because it includes measurement error) were nested within the full ACE model and the best-fitting model selected, as indicated by the lowest absolute value of the AIC and smallest $\Delta\chi^2$. The AIC is also used for comparing nonnested models (i.e., in the case of comparing the fit of ACE and ADE models). Generally, the model with the overall lowest AIC indicates the most parsimonious solution, the best model to explain the structure of the observed data. MLSEM was performed in R (21) by using the structural equation modeling software OpenMx, version 2.2.6 (22).

RESULTS

Participants' mean age was 19.1 y (SD = 0.3 y; range = 18.6–19.6 y), and there were slightly more females (59.8%) than males in our sample. One-third (35.3%) of the twins were monozygotic, the expected proportion of twins who are monozygotic in the United Kingdom general population. The average BMI was 22.3 (SD = 4.2; range = 13.5–59.8), indicating that the sample was relatively lean. A small number reported a vegetarian (n = 120; 4.19%), pescatarian (n = 77; 2.69%), or vegan (n = 20; 0.7%) diet. There were few food allergies. Peanut allergy was the most common (n = 54; 1.88%) followed by tree nuts (n = 34; 1.19%), wheat/gluten (n = 31; 1.08%), and dairy (n = 28: 0.98%). A full overview of sample characteristics is shown in **Table 1**.

All of the foods on the food preference questionnaire had been tried by >85% of the participants. Mean food item preference scores ranged from 2.28 (SD = 1.39) for cottage cheese (the least-liked food) to 4.70 (SD = 0.64) for chocolate (the most-liked food) (**Table 2**). Pearson's correlation coefficients indicated that all food-group preference scores were positively associated and that the strongest positive correlation was seen between fruit and vegetable liking (r = 0.58, P < 0.001). Preference scores for vegetables and snacks had the lowest correlation (r = 0.055, P = 0.003). The ICCs were higher for monozygotic pairs than for dizygotic pairs for all foods, suggestive of genetic influence on variation in liking for all food groups.

Mean food category liking scores are shown in **Table 3**. With a mean preference score of 4.39 (SD = 0.55), snacks were rated as the most popular. In contrast, vegetables were the least-liked group of foods, with a mean preference score of 3.59 (SD = 0.78). In keeping with the patterns of twin correlations for the individual food items, monozygotic pairs were more similar than dizygotic pairs for all 6 food categories, suggestive of genetic influence on food preferences at the group level as well as at the individual food-item level. A broad pattern emerged, which showed that all food category dizygotic within-pair correlations were less than half the monozygotic ICCs, which can indicate the presence of nonadditive genetic factors (D).

Results from the MLSEM provided more detailed insights into the relative influence of genetic and environmental factors on variations in food preferences. In general, liking for individual food items appeared to be almost entirely explained by genetic

TABLE 1
Demographic characteristics of the study sample

	Sample (n = 2865)
Sex, n (%)	
Male	1152 (40.21)
Female	1713 (59.79)
Zygosity, n (%)	
Monozygotic	1010 (35.25)
Dizygotic	1855 (64.75)
Age, y	19.1 ± 0.3[1]
BMI, kg/m^2	22.3 ± 4.2
Diet type, n (%)	
None	2648 (92.42)
Pescatarian	77 (2.69)
Vegetarian	120 (4.19)
Vegan	20 (0.70)
Food allergy, n (%)	
Peanuts	54 (1.88)
Tree nuts	34 (1.19)
Sesame	5 (0.17)
Dairy	28 (0.98)
Shellfish	13 (0.45)
Fish	6 (0.21)
Egg	4 (0.14)
Wheat/gluten	31 (1.08)
Soy	5 (0.17)
Celery	2 (0.07)
Mustard	3 (0.10)
Other[2]	49 (1.71)

[1]Mean ± SD (all such values).
[2]Includes strawberries, oranges, and apples.

influences and the unique environment. For all of the food items, it was possible to drop the shared environmental factor (C), with AE models being preferred in every case. In fact, for almost all foods, the shared environmental effect was estimated to be 0, which indicated no detectable effect of the shared environment on any food preferences in this sample. Heritability estimates for individual food items ranged from 0.18 (95% CI: 0.10, 0.25) for bread to 0.53 (95% CI: 0.46, 0.59) for avocado. The ACE modeling results for each individual food item are presented in full in **Supplemental Table 1**.

The pattern of the ICCs for monozygotic and dizygotic twins for the different food groups suggested the presence of some nonadditive (D) genetic effects. The use of MLSEM to estimate A, D, and E indicated that variation was explained by additive genetic (A) and nonshared environmental (E) effects, with non-additive genetic effects (D) being nonsignificant for most food categories. For 5 of 6 food categories, AE models were found to provide the most parsimonious solution. One exception was fruit, with 15% of preference variation explained by dominant genetic effects (D: 0.15; 95% CI: 0.06, 0.24). A full list of estimates and test statistics can be found in **Supplemental Table 2**. Because this study was underpowered to detect small dominant genetic effects (for the majority of the food categories), ACE models were also considered. Estimates for all ACE models with the full model-fitting results are shown in **Supplemental Table 3**. Similar to the ADE solution, the MLSEM results for the food groups that included C instead of D showed no influence of the shared environment on liking for any food group (**Figure 1**). Again, the best-fitting model for each food group was an AE model, which constrained the shared environmental influence (C) to zero.

ACE and ADE models were compared for goodness-of-fit by using the AIC (23). Both ACE and ADE models provided similar fits to the data, apart from vegetable and starch preferences, which favored ADE models (ΔAIC ≥ 2); ACE and ADE models were of comparable fit for the remaining 4 food categories. Because large sample sizes are needed to detect significant, small, nonadditive genetic effects, we were underpowered to do so. Moderate heritability estimates, obtained from ACE models, were found for liking of most food groups: vegetables (0.54; 95% CI: 0.47, 0.59), fruit (0.49; 95% CI: 0.43, 0.55), meat or fish (0.44; 95% CI: 0.38, 0.51), dairy (0.44; 95% CI: 0.37, 0.50), starches (0.32; 95% CI: 0.24, 0.39), and snacks (0.43; 95% CI: 0.36, 0.49). For each of these food groups, approximately half of the observed variation in preference ratings was accounted for by genetic factors. For all of the food groups the unique environmental effects explained the remaining variance. Significant nonadditive genetic influences (D) were detected for fruit (Supplemental Table 3).

Sensitivity analyses were undertaken to evaluate the impact of self-reported dietary restrictions (e.g., vegetarians or individuals with specific allergies) on ACE estimates for each group. The exclusion of all preference scores for individuals who reported any dietary restrictions (n = 358) did not alter the results for any food group. Thus, observations from these individuals were excluded from the analysis only if relevant to the reported diet type or allergy (e.g., vegans and vegetarians were not included in the analyses of preferences for meat or fish). Full details of the sensitivity analysis (n = 2309) are shown in **Supplemental Table 4**.

DISCUSSION

The present study establishes the relative importance of genetic, shared, and unique environmental influences on variations in food preferences in early adulthood. The results show that early shared environmental factors between siblings (e.g., the household or school setting) do not appear to significantly influence food preferences at this older age. Nonetheless, in keeping with previous pediatric and adult studies, we also observed a moderate genetic influence on food preference variations in young adults.

These findings confirm previous research on the etiology of food preferences, which consistently showed a sizeable genetic influence on individual variations in food preferences or intakes (8, 9, 24). Importantly, our results suggest that significant shared environmental influences from childhood are replaced by unique environmental factors by the time individuals enter young adulthood, although longitudinal data from the same sample are needed to test the assumption that shared environmental influences disappear once individuals are able to make autonomous food choices. This is in line with the results of the only other study, to our knowledge, that has investigated genetic and environmental influences on dietary intakes in a sample of young adults (24). In addition, for adolescents, food encounters increasingly occur outside of the family home. The absence of an enduring shared environmental effect has also been documented for other eating behaviors, for example, food intake patterns (14, 25) and general nutrient intake (26–28). Although these findings are broadly consistent with most food preference research undertaken in adults, a small Danish study in adult twins did find significant influences of the shared environment on dietary intake (12). However, these contradictory estimates were

TABLE 2
Food item preference scores and ICCs by zygosity[1]

Food item	*n* (%)[2]	Mean preference score[3] (SD)	ICC (95% CI) Monozygotic	ICC (95% CI) Dizygotic
Vegetables				
Spinach	2686 (94.01)	3.33 (1.42)	0.472 (0.391, 0.545)	0.076 (0.104, 0.251)
Carrots	2851 (99.79)	4.29 (1.01)	0.305 (0.221, 0.384)	0.076 (0.004, 0.147)
Green beans	2816 (98.56)	3.87 (1.24)	0.303 (0.216, 0.385)	0.021 (0.000, 0.093)
Cucumbers	2843 (99.51)	4.04 (1.29)	0.354 (0.271, 0.431)	0.130 (0.059, 0.199)
Celery	2767 (96.95)	2.86 (1.52)	0.464 (0.387, 0.533)	0.227 (0.157, 0.295)
Mushrooms	2826 (98.91)	3.24 (1.67)	0.459 (0.382, 0.529)	0.148 (0.077, 0.217)
Brussels sprouts	2801 (98.04)	2.75 (1.58)	0.471 (0.395, 0.539)	0.145 (0.074, 0.215)
Parsnips	2774 (97.09)	3.35 (1.54)	0.487 (0.411, 0.555)	0.122 (0.050, 0.193)
Peas	2848 (99.69)	4.06 (1.26)	0.273 (0.184, 0.356)	0.062 (0.000, 0.132)
Sweet corn	2842 (99.48)	4.21 (1.19)	0.319 (0.232, 0.401)	0.090 (0.019, 0.159)
Broccoli	2831 (99.09)	4.03 (1.24)	0.371 (0.291, 0.446)	0.080 (0.008, 0.152)
Salad	2848 (99.69)	4.16 (1.08)	0.335 (0.253, 0.412)	0.057 (0.000, 0.128)
Red peppers	2825 (98.88)	4.02 (1.28)	0.423 (0.344, 0.495)	0.114 (0.041, 0.184)
Raw tomatoes	2840 (99.41)	3.29 (1.66)	0.466 (0.392, 0.535)	0.105 (0.035, 0.175)
Avocados	2455 (85.69)	2.73 (1.50)	0.554 (0.479, 0.621)	0.222 (0.146, 0.295)
Potatoes	2860 (99.83)	4.29 (0.95)	0.317 (0.234, 0.395)	0.069 (0.000, 0.138)
Baked beans	2844 (99.30)	3.94 (1.27)	0.317 (0.230, 0.399)	0.085 (0.015, 0.154)
Beetroot	2681 (93.84)	2.70 (1.58)	0.532 (0.459, 0.597)	0.238 (0.166, 0.307)
Fruit				
Oranges	2857 (99.79)	4.30 (1.01)	0.400 (0.322, 0.473)	0.177 (0.109, 0.244)
Grapes	2855 (99.65)	4.62 (0.82)	0.429 (0.350, 0.501)	0.107 (0.038, 0.175)
Apples	2858 (99.83)	4.54 (0.80)	0.553 (0.000, 0.956)	0.000 (0.000, 0.850)
Melon	2839 (99.09)	4.04 (1.29)	0.342 (0.259, 0.421)	0.139 (0.066, 0.210)
Peaches	2795 (97.56)	3.95 (1.26)	0.489 (0.415, 0.556)	0.231 (0.160, 0.298)
Apricots	2736 (95.50)	3.48 (1.37)	0.381 (0.296, 0.460)	0.205 (0.133, 0.275)
Strawberries	2850 (99.58)	4.53 (0.98)	0.460 (0.387, 0.528)	0.082 (0.013, 0.151)
Meat or fish				
Beef	2630 (95.98)	4.36 (0.99)	0.429 (0.342, 0.507)	0.149 (0.070, 0.226)
Beef burgers	2623 (95.64)	4.35 (1.01)	0.350 (0.250, 0.441)	0.123 (0.044, 0.200)
Lamb	2610 (95.01)	3.92 (1.32)	0.508 (0.429, 0.578)	0.244 (0.169, 0.315)
Chicken	2644 (96.61)	4.80 (0.52)	0.192 (0.090, 0.288)	0.090 (0.006, 0.171)
Bacon	2607 (95.11)	4.46 (0.97)	0.361 (0.266, 0.449)	0.051 (0.000, 0.126)
Ham	2608 (95.18)	4.17 (1.05)	0.382 (0.290, 0.465)	0.115 (0.040, 0.189)
Sausages	2632 (96.09)	4.37 (0.96)	0.323 (0.235, 0.405)	0.067 (0.000, 0.146)
White fish	2700 (96.93)	3.97 (1.29)	0.391 (0.301, 0.473)	0.099 (0.018, 0.178)
Canned tuna	2680 (96.19)	3.68 (1.56)	0.553 (0.482, 0.617)	0.226 (0.152, 0.297)
Oily fish	2555 (91.69)	2.74 (1.51)	0.551 (0.477, 0.616)	0.183 (0.106, 0.257)
Smoked salmon	2608 (93.51)	3.31 (1.61)	0.424 (0.337, 0.502)	0.172 (0.095, 0.246)
Hummus	2497 (87.16)	3.15 (1.56)	0.532 (0.451, 0.602)	0.261 (0.185, 0.333)
Dairy				
Eggs	2822 (99.09)	4.13 (1.25)	0.422 (0.342, 0.495)	0.014 (0.000, 0.088)
Soft cheese	2725 (96.54)	3.34 (1.47)	0.484 (0.406, 0.554)	0.206 (0.134, 0.276)
Hard cheese	2803 (99.37)	4.23 (1.12)	0.292 (0.201, 0.378)	0.064 (0.000, 0.140)
Butter	2794 (99.23)	3.96 (1.09)	0.293 (0.205, 0.376)	0.074 (0.000, 0.147)
Cream	2791 (99.02)	3.74 (1.24)	0.266 (0.171, 0.355)	0.013 (0.000, 0.087)
Yogurt	2758 (97.91)	3.63 (1.22)	0.310 (0.221, 0.392)	0.067 (0.000, 0.142)
Cottage cheese	2525 (89.56)	2.28 (1.39)	0.396 (0.305, 0.477)	0.185 (0.108, 0.259)
Butter-like spread	2832 (99.02)	3.78 (1.14)	0.400 (0.320, 0.474)	0.124 (0.051, 0.196)
Mayonnaise	2816 (98.88)	3.56 (1.43)	0.481 (0.406, 0.549)	0.161 (0.091, 0.229)
Custard	2793 (99.48)	3.93 (1.35)	0.494 (0.420, 0.561)	0.203 (0.133, 0.271)
Snacks				
Chips	2861 (99.86)	4.54 (0.76)	0.339 (0.254, 0.418)	0.071 (0.000, 0.143)
Plain biscuits	2854 (99.79)	4.21 (0.91)	0.329 (0.242, 0.410)	0.200 (0.128, 0.270)
Chocolate biscuits	2854 (99.79)	4.56 (0.76)	0.276 (0.185, 0.361)	0.107 (0.033, 0.180)
Cake	2854 (99.79)	4.51 (0.83)	0.179 (0.087, 0.268)	0.140 (0.069, 0.210)
Ice cream	2851 (99.69)	4.52 (0.81)	0.293 (0.207, 0.375)	0.095 (0.024, 0.164)
Chocolate	2855 (99.83)	4.70 (0.64)	0.277 (0.191, 0.358)	0.076 (0.002, 0.150)
Crisps	2855 (99.83)	4.46 (0.83)	0.362 (0.279, 0.439)	0.114 (0.042, 0.185)

(Continued)

TABLE 2 (*Continued*)

Food item	*n* (%)[2]	Mean preference score[3] (SD)	ICC (95% CI) Monozygotic	ICC (95% CI) Dizygotic
Gummy sweets	2833 (99.06)	4.12 (1.11)	0.420 (0.340, 0.493)	0.152 (0.082, 0.221)
Sugared cereal	2851 (99.51)	3.92 (1.12)	0.347 (0.262, 0.426)	0.206 (0.137, 0.273)
Starches				
Bread	2859 (99.83)	4.49 (0.74)	0.193 (0.102, 0.281)	0.070 (0.000, 0.141)
Bran cereal	2790 (97.42)	3.41 (1.24)	0.399 (0.316, 0.476)	0.107 (0.034, 0.177)
Porridge	2811 (98.15)	3.50 (1.36)	0.452 (0.376, 0.521)	0.094 (0.022, 0.165)
Rice	2851 (99.51)	3.95 (1.04)	0.300 (0.215, 0.380)	0.088 (0.016, 0.158)
Wheat cereal	2816 (98.99)	3.98 (1.09)	0.303 (0.217, 0.384)	0.105 (0.033, 0.176)
Rice or corn cereal	2854 (99.62)	4.03 (1.00)	0.292 (0.204, 0.375)	0.090 (0.017, 0.162)

[1]ICC, intraclass correlation.
[2]"*n*" indicates the number of observations included in the mean food liking score (excluding observations from individuals who reported a restrictive dietary requirement). Percentages reflect the full sample who reported trying the item.
[3]Preference scores were rated on a 5-point Likert scale, with a higher score indicating a higher preference for the food item.

derived from food intake data collected by using 1-mo dietary recalls, which is likely to be affected by social desirability bias and have wide 95% CIs.

People intuitively think of cultural influences as playing an important role in shaping food preferences, and many of these cultural influences—both those at the smaller family level as well as at the wider societal level, such as national cuisines—are shared by twin pairs. Finding a substantial influence of the unique environment on food preferences was therefore surprising. However, this observation suggests that twin pairs respond differently to the cultural influences that they are both exposed to. This supports a wealth of research highlighting that children who grow up within the same family experience the same environmental exposures differently (29). Definitive evidence of this developmental change requires a longitudinal study in which the same sample is compared in childhood, adolescence, and adulthood.

The findings of genetic influences on food preferences replicated the results from a previous study in a sample of 3-y-old twins (8). This suggests that food preferences appear early in life and can be reliably measured and that genetic influences on these affinities remain stable over time. The similarities of our food preference heritability estimates compared with this previous study are shown in **Supplemental Figure 1**. Although our results suggest that shared environmental effects detected in early childhood may disappear by late adolescence, the genetic and environmental influences on these traits have not been studied in the same sample at both ages. It is therefore possible that the different estimates of the influence of the shared environment on food preferences could reflect other factors, such as cohort effects.

A substantial heritability of food preferences does not preclude the potential for environmental modification, especially alongside a sizeable environmental influence. Experimental research has shown that repeated exposure to tastes increases flavor acceptance, showing that environmental modification is possible (30–34). To our knowledge, no research has yet investigated the effectiveness, acceptability, or feasibility of a taste modification program in an adult population.

The environment was an important source of influence on food liking in this study (and others), but established drivers of actual food intake, such as cost, availability, and self-regulation, which may also influence food liking, were not accounted for in this study. The ICCs for both the individual food items and the food category scores (Tables 2 and 3) were suggestive of nonadditive genetic effects, because the dizygotic ICCs were less than half the monozygotic ICCs. However, we were unable to detect significant estimates for D (shown in Supplemental Table 3), because

TABLE 3
Food category preference scores, ICC scores by zygosity, and ACE variable estimates from the model of best fit for the 6 food groups[1]

Food item	*n*	Mean preference score (SD)	ICC (95% CI) Monozygotic	ICC (95% CI) Dizygotic
Vegetables	2865	3.59 (0.78)	0.575 (0.511, 0.632)	0.169 (0.096, 0.237)
Fruit	2862	4.19 (0.80)	0.518 (0.449, 0.581)	0.232 (0.163, 0.298)
Meat or fish	2855	3.89 (0.77)	0.450 (0.374, 0.520)	0.183 (0.110, 0.253)
Dairy	2865	3.62 (0.73)	0.471 (0.399, 0.538)	0.157 (0.086, 0.229)
Snacks	2860	4.39 (0.55)	0.460 (0.385, 0.529)	0.154 (0.082, 0.224)
Starches	2864	3.88 (0.70)	0.362 (0.279, 0.438)	0.084 (0.012, 0.154)

[1]Standard ACE model-fitting analyses for continuous data were used. The full ACE model was nested within the saturated model, with subsequent submodels nested within the full ACE model. The selection of the most parsimonious model was indicated by the lowest absolute value of the ACE and the smallest $\Delta\chi^2$. Full model-fitting results are summarized in Supplemental Table 2. ACE, Akaike information criterion; ICC, intraclass correlation.

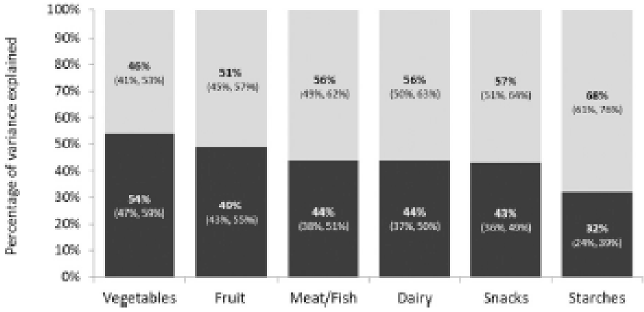

FIGURE 1 Genetic and environmental influences on food preference categories. Estimates of the percentages in food preference variation explained by genetic factors shown in this figure are based on 2865 participants of the TEDS (Twins Early Development Study) twin cohort. Food preference data were ascertained by self-report with the use of a food preference questionnaire when the participants were 18–19 y old. Genetic influences ("A"), black portion of bars; unique environment influences ("E"), gray portion of bars.

we were underpowered to detect these small effects. Nevertheless, given that the D effects were generally small, AE models were a fair representation of the data. Furthermore, liking scores for popular food items such as chocolate were high, with mean scores of <1 SD below the maximum, which may have limited heritability estimates (7). Nonetheless, the food preference measures were able to capture sufficient variance, and the groups with possible ceiling effects did not show significantly different results from the others. Because these findings were established in a predominantly white British and lean twin sample, the extent to which these results may be generalized to the population as a whole may be limited.

However, the large sample size and narrow age range are strengths that allowed reliable estimates for food preferences to be established for a specific developmental phase. A limitation of existing studies in adults is the very wide age range included in each analysis, with studies typically including individuals from early adulthood to older age, making it impossible to ascertain if influences are different for younger and older adults.

It is well established that sweet tastes are universally accepted, and bitter tastes disliked. These dispositions are thought to be the artifacts of an evolutionary adaptive process that facilitated the identification of safe sources of dietary energy and the avoidance of potentially toxic substances (35). However, there is considerable population variation in these preferences, and there is now some molecular genetic evidence to support the heritability estimates observed for variations in some food preferences. Polymorphisms in the Taste 2 receptor gene family (*TAS2R*) genes, a family of 25 bitter-taste receptors, have been associated with variations in sensitivity toward bitter-tasting compounds, such as phenylthiocarbamide (36) and 6-n-propylthiouracil (37, 38). Individuals with a copy of the dominant "taster" PAV haplotype (PAV denotes the encoded amino acid sequence) in the *TAS2R38* gene are most sensitive to 6-n-propylthiouracil (38), and a number of studies have associated lower liking of cruciferous vegetables with this genotype (39, 40). However, more research is needed to identify genetic variants associated with other taste preferences, such as sweet preference. There may also be psychological traits that underlie food preferences. A recent study in 3-y-old British twins established that a substantial proportion of the genetic influence on fruit and vegetable liking could be explained by the genetic influence on food fussiness (41).

Other genetic variants have been identified that are associated with variations in taste perception, but their modes of action are largely unknown. Possible mechanisms include the following: taste receptor density on the tongue (42), reward circuitry (43), and cognitive processes related to self-regulation (44), extraversion (45), food neophobia (46), or anxiety (47), all of which have been associated with food preferences (48).

These results suggest that food preferences are a reasonable target for DNA research. Further research is needed to characterize the biological pathways from genes to behavior. On the other hand, we know a reasonable amount about the environmental shapers of taste preferences.

Food preferences of older adolescents are influenced by both genetic and unique environmental influences. However, our findings of no significant effect of the shared environment on food preferences in adolescence could suggest that children's early shared family experiences relating to food preferences may not have lasting effects. Overall, our findings indicate that food preferences are approximately equally influenced by genetic, and nonshared, environmental factors. Efforts to improve adolescent nutrition may, for that reason, be best targeted at the wider environment. Strategies might include increasing the availability, lowering the cost, of and promoting "healthier foods" (49). This approach requires stronger government legislation and regulation of the food environment (50).

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The authors' responsibilities were as follows— ADS, AF, RP, and CL: designed the research; RP: provided essential materials; ADS and NS: conducted the research; ADS and MH: performed the statistical analysis; ADS, AF, LC, MH, and CL: wrote the manuscript; ADS: had primary responsibility for the final content; and all authors: read and approved the final manuscript. None of the authors reported a conflict of interest related to the study.

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Appendix G3 Published paper on the aetiology of drink preferences (Chapter 5)

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SCIENTIFIC REPORTS

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The individual environment, not the family is the most important influence on preferences for common non-alcoholic beverages in adolescence

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Beverage preferences are an important driver of consumption, and strong liking for beverages high in energy (e.g. sugar-sweetened beverages [SSBs]) and dislike for beverages low in energy (e.g. non-nutritive sweetened beverages [NNSBs]) are potentially modifiable risk factors contributing to variation in intake. Twin studies have established that both genes and environment play important roles in shaping food preferences; but the aetiology of variation in non-alcoholic beverage preferences is unknown. 2865 adolescent twins (18–19-years old) from the Twins Early Development Study were used to quantify genetic and environmental influence on variation in liking for seven non-alcoholic beverages: SSBs; NNSBs; fruit cordials, orange juice, milk, coffee, and tea. Maximum Likelihood Structural Equation Modelling established that beverage preferences have a moderate to low genetic basis; from 18% (95% CI: 10%, 25%) for orange juice to 42% (36%, 43%) for fruit cordials. Aspects of the environment that are *not* shared by twin pairs explained all remaining variance in drink preferences. The sizeable unique environmental influence on beverage preferences highlights the potential for environmental modification. Policies and guidelines to change preferences for unhealthy beverages may therefore be best directed at the wider environment.

Beverages are increasingly becoming substantial contributors to individual energy intake as the availability and diversity of sugar-sweetened beverages (SSBs), fruit juices and other calorie-containing beverages continues to grow[1]. There is considerable concern about the increased consumption of SSBs, mainly because calorically-sweetened liquids have a weak satiety effect, which may result in poor energy compensation, and the development of overweight[2]. Negative health consequences are not limited to an increased risk of obesity, but also include the comorbidities associated with obesity – such as type 2 diabetes, cardiovascular disease and raised risk for various cancers – and dental caries. Global beverage consumption patterns have evolved in the past decades with a marked increase in the consumption of SSBs, although it appears that the market share for non-nutritive sweetened beverages (NNSBs) is gradually growing in importance. Purchases of lower sugar, zero calories soft drinks have for the first time overtaken regular soft drinks as the most popular soft drink type in the UK[3]. Nevertheless, the overarching shift towards greater consumption of SSBs, fruit juices and energy drinks among adolescents, in particular in low- to middle-income countries, highlights this as a key population at risk of the detrimental health risks associated with frequent consumption of energy-dense beverages[4–6].

Preferences are important drivers of actual intake insofar as we tend to eat and drink what we like most. Stronger liking for beverages high in energy and decreased liking for beverages low in energy may therefore be important contributors to variation in intake. Preference for energy-dense beverages during adolescence may be especially detrimental to energy balance as consumption of these beverages peaks during this developmental period[7].

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Understanding what shapes preferences for different types of beverages is a crucial first step towards the development of interventions to modify them[8]. Twin studies are a powerful method for understanding the aetiology of behaviors and cognitions, because they establish the relative importance of genetic and environmental influences on variation in any given trait. Previous twin studies have established that individual differences in preferences for a range of foods have a moderate genetic basis in children (54% for vegetables, 27% for dairy and protein foods, and 20% for dessert foods)[9,10], adolescents (54% for vegetables, 43% for snack foods, 54% for 'healthy' foods, and 39% for meat)[11,12] and adults (30–38% for high fat, salt and sugar foods, 40% for 'healthful' foods, 58% for 'distinctive' tastes, and 36% for fruit and vegetables)[13,14]; yet the aetiologies of preferences for a variety of non-alcoholic beverages are yet to be established. Two previous twin studies examined genetic and environmental influences on preferences for coffee[15,16], finding moderate genetic influence (42% in Luciano *et al*., 2005 and 62% in Vink *et al*., 2009). However, both studies defined coffee preference as a relative preference to tea, indexed as the ratio of number of cups of coffee to tea, consumed per day, rather than absolute liking of coffee per se.

Food and beverage preferences may differ substantially with respect to their aetiology. There is a need for studies to investigate the relative contribution of genetic and environmental factors to variation in preferences for a range of different types of beverages to inform where best to direct public health initiatives aimed at decreasing consumption of energy-dense beverages, especially among adolescents. The objective of this study was therefore to establish the relative importance of genetic and environmental influences on preferences for a range of non-alcoholic beverages among older adolescents.

Subjects and Methods

Sample.

Participants were drawn from the Twins Early Development Study (TEDS), a population-representative cohort of 16,810 families with twins born in England and Wales in 1994–96[17]. The TEDS sample is largely representative of the UK population[17]. For the current study, twins were from a subsample of pairs born between September 1995 and August 1996. Invitations to complete an online beverage preference questionnaire were sent by letter and e-mail to the entire sub-sample (3166 pairs; n = 6332 individuals). Participation was rewarded with a £10 voucher to complete the survey and entry into a prize draw to win a pair of iPad Minis. Entry into the prize draw was conditional on completion of the questionnaire by the co-twin, to encourage responses from complete twin pairs. Participants provided informed consent. Procedures followed were in accordance and approved by the King's College London Institute of Psychiatry ethics committee.

3155 of 6332 invited (49.8%) individuals agreed to participate in the current study. Of those, 290 were excluded due to medical- or perinatal problems, or due to unknown sex or zygosity. The final sample consisted of 2865 individuals, representing 1010 monozygotic twin individuals, 908 dizygotic same-sex (DZss) twin individuals, and 947 DZ opposite-sex (DZos) individuals. Of the excluded participants, 52 (17.9%) were MZs, 156 (58.6%) were DZs and 82 (21.8%) were of unknown zygosity. Excluded individuals did not differ significantly by zygosity ($F(1, 3496) = 7.01$, $p = 0.08$) or for factors that may be linked with beverage preferences such as health status ($\chi^2 = 5.918$, $p = 0.15$) or BMI ($t = 0.45$, $p = 0.65$).

Measures

Sociodemographic measures and zygosity.

Basic demographic information had been collected at first contact (age 18 months), including data on date of birth, sex, birth- or medical complications and socioeconomic status. Participants reported current height and weight, which was used to compute body mass index (BMI), calculated by dividing weight by height squared (kg/m^2). Zygosity of same-sex twin pairs had previously been assigned using a parent-rated similarity questionnaire, validated by DNA analysis and shown to be >95% accurate[18]. Pairs for whom zygosity was uncertain had their zygosity determined by DNA genotyping, if DNA was available.

Beverage preferences.

As part of a wider study focused on studying food preference patterns in adolescence, seven beverage types were included in a comprehensive online self-report food preference questionnaire. Participants were instructed to rate their liking for sugar-sweetened beverages (SSBs) ('non-diet fizzy beverages (e.g. Coca Cola, Pepsi)'), non-nutritive sweetened beverages (NNSBs) ('diet fizzy beverages (e.g. Diet Coke, Pepsi Max)'), fruit cordial ('Ribena or other fruit squash (e.g. orange squash)'), 'orange juice', 'milk', 'tea (unsweetened)', and 'coffee (unsweetened)' on a 5-point Likert scale, anchored by 'not at all' to 'a lot'. For the 'tea' and 'coffee' items, separate questions enquired about preferred add-ins (e.g. addition of milk or any type of sweetness), but these preferences were not included in the present study. Higher scores indicated greater liking of that type of beverage. Paired-samples t-tests were used to compare mean group preferences for all seven beverage types. For any beverage types that had never been tasted or were unfamiliar, participants were instructed to select a 'Not applicable' option.

Test-retest reliability of the beverage preference questionnaire was assessed over a two-week period using a sample of the twins' siblings. Of the 205 siblings invited to complete the online test-retest of the beverage questionnaire, 94 siblings provided responses at both rounds of data collection. Mean beverage preference test-retest scores indicated that responses were reliable over a 2-week period, with test-retest coefficients as follows: 0.69 for tea, 0.72 for orange juice, 0.77 for coffee, 0.85 for milk, 0.86 for SSBs, 0.89 for fruit cordial, and 0.93 for NNSBs.

Statistical analysis.

Twin analyses were used to estimate genetic and environmental influence on variation in preference for each of the seven beverage types.

The basis of the twin method is to compare the degree of resemblance between identical (monozygotic, MZ) pairs who share 100% of their genes, with that between non-identical (dizygotic, DZ) pairs who share approximately 50% of their segregating genes. The more similar MZ pairs are for a trait than DZ pairs, the stronger the genetic contribution to that trait. This inference is based on the assumption that MZ twins are twice as similar

Characteristic	Sample [n (%)]	
Sex		
M	1152	(40.2)
F	1713	(59.8)
Zygosity		
MZ[1]	1010	(35.3)
DZ[1]	1855	(64.7)
Age (SD)	19.1	(0.3)
BMI (SD)	22.3	(4.2)

Table 1. Demographic characteristics of the study sample (n = 2865). [1]Abbreviations: MZ = Monozygotic; DZ = Dizygotic.

genetically compared to DZs, but both types of twins share their environments to a very similar extent[19]. The statistic derived to estimate the genetic contribution is called 'heritability' (A; additive genetic influences), and can be thought of as an index of the genetic effect size; heritability quantifies the proportion of trait variation attributable to genetic variation. As well as estimating genetic influence, the twin method partitions sources of environmental influence into: (i) aspects that are completely shared by both twins and contribute to their similarity, such as the home environment, SES and schooling experiences ('shared environmental influences'); and (ii) and aspects that are unique to each twin in a pair and contribute to differences between pairs, such as having different friends or bouts of illness experienced by only one of the twins ('unique environmental influences'). Variation is therefore attributed to three components of variance: additive genetic influences (A), shared environmental influences (C) and non-shared environmental influences (E). The unique environmental component of variance also includes random measurement error.

Intra-class correlations (ICCs) were calculated for beverage preference scores for MZ and DZ pairs to provide an indication of the pattern of similarity for the two types of twins. Maximum Likelihood Structural Equation Modelling (MLSEM) was used to derive precise estimates of A, C and E (with 95% confidence intervals and goodness-of-fit statistics) based on the expected structure of the variance-covariance matrices for MZs and DZs, based on the assumptions of genetic relatedness and equal environments (e.g. MZ covariation reflects the fact that they share all of their genes and all of their shared environments, $rMZ = 1A + 1C$; on the other hand, DZ covariation reflects the fact that they share half of their genes, but all of their shared environments, $rDZ = 0.5A + 1C$). Non-additive genetic effects, denoted by 'D', can also be investigated in a separate model that includes D instead of C (A, D and E), but D and C cannot be estimated in the same model with twins only. MZ ICCs that are greater than twice the DZ ICCs ($ICC_{MZ} > 2\ ICC_{DZ}$) indicate non-additive genetic effects contributing to variation. Because beverage preference scores were skewed, an alternative approach to deal with such variables is to summarize preference scales and to dichotomize them by the median value of the scale. Instead of ICC's, tetrachoric correlation coefficients (TTC's) are calculated to estimate phenotypic concordance rates for both types of twins.

Preference ratings for each of the beverage types were regressed on age and sex prior to modelling because twins share their age exactly (and sex for same-sex twins), and these factors can therefore inflate the shared environmental effect[20]. Prior to estimating A, C and E, a saturated model was fitted which applies no constraints to the data, and simply estimates means, covariances and variances for MZs and DZs. The model specifying A, C and E (ACE model) was then compared to the saturated model for goodness-of-fit, using the Likelihood Ratio Test (LRT) and the Akaike Information Criterion (AIC). According to the LRT a significant change in fit from the saturated model to a specified model indicates worsening of fit; a non-significant change indicates that the ACE model fits the data well (i.e. no significant change in fit). A lower AIC value indicates a better fitting model, with a difference of >2 indicating a superior model (AIC was also used for comparing non-nested models; e.g. to compare an ACE to an ADE model). Submodels that dropped A or C or A and C were then tested against the full ACE model to find a more parsimonious model. Just as for continuous traits, a liability threshold model was used to estimate A, C, and E for categorical (i.e. dichotomized) data. Sex-limitation models were also tested for each beverage type, to establish if there were sex-specific effects. These models test whether the magnitude of A, C and E differ for males and females (*quantitative sex-differences*), and whether the genetic and environmental influences are the same or different for males and females (*qualitative sex differences*)[21]. MLSEM was performed in R[22], using the structural equation modelling software OpenMx, version 2.2.6[23]. The datasets generated during and analysed during the current study are available from the corresponding author on reasonable request.

Results

Characteristics of the sample are shown in Table 1. Mean age of the sample was 19.1 years (SD = 0.3), and the sample was reasonably lean (mean BMI = 22.3 kg/m^2). 40.2% of participants were male, and the MZ/DZ ratio (MZ pairs = 35.3%) reflected that of the general European twin population (roughly 1:2)[24].

Table 2 summarizes mean preference scores for each beverage type. Orange juice was the most popular beverage, with the highest mean preference score, and NNSBs were the least liked indicated by the lowest mean score. There were significant differences in mean preference scores across all beverage pairings ($p < 0.01$) apart from between liking for fruit cordial ([4.23 (SD = 1.02)] and milk [4.22 (SD = 0.95)], $t(2702) = 0.166$, $p = 0.87$).

The MZ and DZ intraclass correlations for the seven beverage types are also shown in Table 2. For each beverage type within-pair correlations (ICCs) for MZs were moderate and higher than for DZs, indicating a genetic

Beverage item	n[1] (%)[2]	Mean preference score[3] (SD)	MZ[4] ICC[4] (95% CI)		DZ[4] ICC[4] (95% CI)	
SSBs[4]	2841 (99.2)	3.73 (1.37)	0.383	(0.301, 0.458)	0.155	(0.085, 0.223)
NNSBs[4]	2827 (98.7)	3.64 (1.34)	0.393	(0.221, 0.384)	0.222	(0.155, 0.288)
Orange juice	2849 (99.4)	4.43 (0.97)	0.261	(0.174, 0.345)	0.000	(0.000, 0.051)
Fruit cordial	2847 (99.3)	4.23 (1.02)	0.422	(0.344, 0.494)	0.212	(0.142, 0.280)
Milk	2707 (94.5)	4.22 (0.95)	0.377	(0.289, 0.457)	0.091	(0.013, 0.168)
Tea	2415 (84.3)	4.31 (1.08)	0.532	(0.450, 0.604)	0.000	(0.000, 0.066)
Coffee	1905 (66.5)	3.85 (1.29)	0.341	(0.214, 0.453)	0.069	(0.000, 0.172)

Table 2. Beverage preference scores and intraclass correlations (ICC) by zygosity. [1]Number of observations included in mean beverage liking score (excl. observations from individuals that never consume the specific beverage). [2]Percentage of the full sample that reported occasional consumption of the beverage. [3]Preference scores were rated on a 5-point Likert scale, with a higher score indicating a higher preference for the beverage item. [4]Abbreviations: ICCs: Intraclass Correlations; MZ: Monozygotic; DZ: Dizygotic; NNSBs: Non-nutritive sweetened beverages, SSB: Sugar-sweetened beverages.

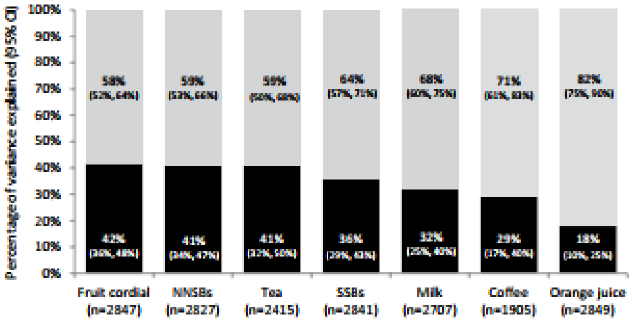

Figure 1. Genetic and environmental influences for the preference of seven non-alcoholic beverages. [1]Estimates of the percentage of variance in beverage preferences explained by genetic (black portions of bars) and environmental (gray portions of bars) factors in 2865 participants from the Twins Early Development Study, aged 18–19 years.

contribution to preferences for each type of beverage. For liking of SSBs, orange juice, milk, tea, and coffee the MZ ICCs were more than twice the magnitudes of the DZ ICCs.

MLSEM was used to derive A, C and E for each beverage type. Heritability estimates (A) across all beverage types were moderate to low for each of: fruit cordial (0.42; 95% CI: 0.36, 0.43), NNSBs (0.41; 95% CI: 0.34, 0.47), tea (0.41; 95% CI: 0.32, 0.50), SSBs (0.36; 95% CI: 0.29, 0.51), milk (0.32; 95% CI: 0.25, 0.40), coffee (0.29; 95% CI: 0.17, 0.40), and orange juice (0.18; 95% CI: 0.10, 0.25). The 95% confidence intervals demonstrated that genetic influences were significantly higher for liking of SSBs, NNSBs, tea and fruit cordial, than for liking of orange juice. No significant influence of the shared environment was observed for liking of any of the beverages, with the remaining variance being explained by environmental effects unique to each individual twin. AE models (that dropped the C component of variance) were therefore preferred for each beverage type. The relative contribution of genetic and environmental influences on individual beverage preferences are shown in Fig. 1, and the ACE model results with goodness-of-fit statistics are shown in Table 3.

Because the MZ ICCs were greater than twice the DZ ICCs for SSBs, orange juice, milk, tea, and coffee preference scores, suggestive of some non-additive genetic effects, ADE models were also examined and compared to ACE models (Supplemental Table 1). For liking of orange juice, milk and tea, the ADE model provided a better fit than the ACE model ($\Delta AIC = 7.8$, 4.07 and 24.19 respectively), but no significant non-additive genetic effects (D) were found. AE models therefore provided the most parsimonious solutions across all beverage types. Similarly, because scale scores were skewed for orange juice, milk, tea and coffee, threshold models (treating beverage preference scores as binary traits) were considered as well as the models of continuous data. However, the genetic and environmental aetiology estimates from the threshold models did not differ significantly from the results shown in Table 3. Due to the loss in statistical power to estimate genetic variance components accurately when a trait is modelled as a binary rather than a continuous variable, results from the liability threshold models are shown in the supplemental materials (Supplemental Tables 2 and 3).

Similarly, results from the sex-limitation models found no strong evidence of significant sex differences. Parameters estimates and goodness-of-fit statistics for the full qualitative and quantitative sex-limitation models are shown in Supplemental Tables 4–11.

Beverage type	Additive genetic effect (A)	Shared environment effect (C)	Nonshared environment effect (E)	-2LL[3]	Df[3]	AIC[3]	Δ -2LL	p-value
SSBs[3]								
Sat				9609.825	2832	3945.825		
ACE[1]	0.36 (0.26, 0.43)	0.00 (0.00, 0.09)	0.64 (0.57, 0.71)	9614.843	2835	3944.843	5.018	0.170
AE[2]	0.36 (0.29, 0.43)	—	0.64 (0.57, 0.71)	9614.843	2836	3942.843	0	1
CE[2]	—	0.24 (0.19, 0.29)	0.76 (0.71, 0.81)	9631.547	2836	3959.547	16.704	< 0.001
E[2]	—	—	1.00 (1.00, 1.00)	9703.572	2837	4029.572	72.025	< 0.001
NNSBs[3]								
Sat				9545.841	2818	3909.841		
ACE[1]	0.35 (0.15, 0.47)	0.05 (0.00, 0.20)	0.60 (0.55, 0.68)	9546.322	2821	3904.322	0.481	0.923
AE[2]	0.41 (0.34, 0.47)	—	0.59 (0.53, 0.66)	9546.719	2822	3902.719	0.397	0.529
CE[2]	—	0.28 (0.23, 0.33)	0.72 (0.67, 0.77)	9557.576	2822	3913.576	11.254	< 0.001
E[2]	—	—	1.00 (1.00, 1.00)	9660.699	2823	4014.699	114.377	< 0.001
Orange juice								
Sat				7844.431	2840	2164.431		
ACE[1]	0.18 (0.09, 0.25)	0.00 (0.00, 0.04)	0.82 (0.75, 0.90)	7862.541	2843	2176.541	18.11	< 0.001
AE[2]	0.18 (0.10, 0.25)	—	0.82 (0.75, 0.90)	7862.541	2844	2174.541	0	1
CE[2]	—	0.08 (0.03, 0.14)	0.92 (0.86, 0.97)	7873.266	2844	2185.266	10.725	0.001
E[2]	—	—	1.00 (1.00, 1.00)	7881.523	2845	2191.523	18.982	< 0.001
Fruit cordial								
Sat				8031.215	2838	2355.215		
ACE[1]	0.42 (0.23, 0.48)	0.00 (0.00, 0.15)	0.58 (0.52, 0.90)	8034.727	2841	2352.727	3.512	0.319
AE[2]	0.42 (0.36, 0.48)	—	0.58 (0.52, 0.64)	8034.727	2842	2350.727	0	0.998
CE[2]	—	0.29 (0.24, 0.34)	0.71 (0.66, 0.76)	8051.672	2842	2367.672	16.945	< 0.001
E[2]	—	—	1.00 (1.00, 1.00)	8157.514	2843	2471.514	122.787	< 0.001
Milk								
Sat				7269.105	2698	1873.105		
ACE[1]	0.32 (0.25, 0.40)	0.00 (0.00, 0.06)	0.68 (0.60, 0.75)	7281.350	2701	1879.350	12.245	0.007
AE[2]	0.32 (0.25, 0.40)	—	0.68 (0.60, 0.75)	7281.350	2702	1877.350	0	1
CE[2]	—	0.20 (0.14, 0.26)	0.80 (0.74, 0.86)	7298.526	2702	1894.526	17.176	< 0.001
E[2]	—	—	1.00 (1.00, 1.00)	7340.874	2703	1934.874	59.524	< 0.001
Tea								
Sat				7098.183	2406	2286.183		
ACE[1]	0.41 (0.32, 0.50)	0.00 (0.00, 0.03)	0.59 (0.50, 0.68)	7134.002	2409	2316.002	35.82	< 0.001
AE[2]	0.41 (0.32, 0.50)	—	0.59 (0.50, 0.68)	7134.002	2410	2314.002	0.00	1
CE[2]	—	0.19 (0.12, 0.26)	0.81 (0.74, 0.88)	7171.829	2410	2351.829	37.83	< 0.001
E[2]	—	—	1.00 (1.00, 1.00)	7201.412	2411	2379.412	67.41	< 0.001
Coffee								
Sat				6351.94	1896	2559.94		
ACE[1]	0.29 (0.12, 0.39)	0.00 (0.00, 0.11)	0.71 (0.61, 0.83)	6358.921	1899	2560.921	6.9809	0.07
AE[2]	0.29 (0.17, 0.40)	—	0.71 (0.61, 0.83)	6358.921	1900	2558.921	0	1
CE[2]	—	0.17 (0.09, 0.25)	0.83 (0.75, 0.91)	6366.625	1900	2566.625	7.7045	< 0.001
E[2]	—	—	1.00 (1.00, 1.00)	6382.128	1901	2580.128	23.208	< 0.001

Table 3. Model fit and parameter estimates for the saturated, ACE model and submodels of beverage preferences. Maximum Likelihood Structural Equation Modelling (MLSEM) was used to derive estimates of A, C and E, as well as provide two goodness-of-fit statistics; -2LL and the AIC respectively. The selection of the most parsimonious model was indicated by the p-value and the lowest absolute value of the AIC. [1]The full ACE model was nested within the saturated model. [2]Sub-models were nested within the full ACE model. [3]Abbreviations; - 2LL: *-2 times log-likelihood of data, df: degrees of freedom, AIC: Akaike Information Criterion (AIC), NNSBs: Non-nutritive sweetened beverages, SSB: Sugar-sweetened beverages.*

Discussion

Summary of findings. This is the first paper to establish the relative importance of genetic and environmental influences on liking for a range of non-alcoholic beverages, in a large population-based sample of older adolescent twins. Liking for SSBs, NNSBs, fruit cordial, orange juice, milk, coffee, and tea are entirely shaped by genetic and *unique* environmental influences in this age group. There was no detectable influence of the shared environment, which includes aspects of the environment completely shared by twin pairs; such as growing up in the same home, having the same parents, or shared schooling experiences. This suggests that shared environmental factors are unimportant in shaping preferences for non-alcoholic beverages for older adolescents, a developmental period characterised by a marked increase in autonomy in relation to dietary behaviour.

Similar to previous studies on the aetiology of food preferences, variation for the liking of seven beverage types was found to have a small to moderate genetic basis. Two previous studies have investigated the genetic and environmental contributions to *relative* liking for coffee (over tea), showing that approximately 42% to 62% of variation in coffee preference was attributable to genetic differences between adults[15,16]. These estimates are at the higher end of those observed in the current study (ranging from 18% for orange juice to 42% for fruit cordial). The preference ratings for coffee likely encompass genetic sensitivity to, and enjoyment of, the stimulation provided by caffeine. However, not only coffee but many other carbonated fizzy beverages contain caffeine, a psychoactive substance that may be a key driver of beverage liking. Gareth and Griffins (1998) suggested the liking for fizzy caffeinated beverages may be reinforced by the invigorating effect of caffeine, and avoidance of withdrawal symptoms, rather than an affinity for the actual flavour of the beverage[25]. These studies suggest that both direct genetic variability in the biological caffeine-response (e.g. polymorphisms in the caffeine metabolism pathway), and the more indirect psychological mechanisms (e.g. sensitivity to caffeine-induced anxiety) might be influencing caffeinated beverage preference scores[26].

In line with the heritability estimates for SSBs, NNSBs, and fruit cordial observed in the present study, one previous twin study showed variation in liking for 'sweet and high carbohydrate foods' which included diet-and non-diet soft beverages and fruit juices, to be moderately heritable (52%) in a large sample of adults ($n = 2596$)[14]. However, this estimate only provides limited comparability due to sweet foods and beverages being combined in this category.

Sweetness dominates as the underlying taste of SSBs, NNSBs, fruit cordial, orange juice, and to some extent for the natural sweetness of milk. Finding moderate genetic influence on liking for non-alcoholic beverages may in part reflect genetically-determined sensitivity or preference for sweetness. A previous twin study found that sweetness sensitivity for two different sugars (glucose and fructose) and two different non-nutritive sweeteners (aspartame and neohesperidine dihydrochalcone), was largely explained by a common genetic pathway underlying them all; this suggests a genetically-determined predisposition towards liking for sweet flavours in general[27]. In addition, a family study found that the pleasantness ratings for a strong sweet solution (41%), the pleasantness rating (40%) and frequency of consumption (50%) of sweet foods, and craving for sweet foods (31%) were all moderately heritable[28]. To date no genome-wide association study has been undertaken to identify common genetic variants associated with sweetness preference, but this is likely the result of the limited availability of data on sweetness preference measures in large samples with genome-wide genotyping. However, polymorphisms in the sweet taste receptor genes T1R2 and T1R3 have been associated with variation in sugar consumption[29] and sucrose taste sensitivity[30]. Evidence from a mouse study suggests that sweetness perception is reliant on the functionality of taste receptor subunits T1R1/T1R3. Zhao *et al.* (2003) observed that mice knockout models for these genes fail to respond to stimulation by sweetness[31], implicating genetic variability in these genes as plausible contributors to the observed genetic basis of beverage preference scores.

The liking scores for tea and coffee in this study enquired about their unsweetened variety, meaning that these were the only non-sweet beverages considered. Evidence from a recent twin study ($n = 1901$; mean age: 16.2 y) found considerable correlations ($r = 0.35–0.40$) between the mean perceived intensity of a "sweet factor" (comprising intensity ratings for glucose, fructose, neohesperidine dihydrochalcone, and aspartame) and perception intensity of three bitter solutions (sucrose octa-acetate, quinine, and caffeine). The correlations were largely explained by shared genetic influences, with 8% of sweetness perception and 17–37% of the bitterness perception ratings attributable to genes common to both traits. In addition, the magnitude of the heritability of sweetness perception (36%) and bitterness perception (35–40%) were at a similar level[32]. The results from this study agree with our findings that heritability estimates did not differ significantly between sweet beverage types (18–42% for SSBs and NNSBs) and non-sweet types (29–41% for tea and coffee). However, it is important to note that the sample sizes were slightly lower for coffee and tea as the mean scores for this group only included those participants who reported drinking these hot beverages, and this may have influenced the heritability estimates for the liking of these non-sweet beverages.

Variation in preferences for beverages was influenced strongly by individual environmental factors (e.g. friends, lifestyle choices), and less so by genetic factors (shared environmental factors were undetectable). This was especially the case for orange juice, coffee and milk, for which 82%, 71%, and 68% of variation in preference scores were explained by unique environmental factors, respectively. The strong influence of the unique (versus shared) environment, reflects the lifestyle changes experienced by adolescents. Adolescence is a period of transition towards independence and autonomy, with greater time spent interacting with peers outside the home, and conforming to perceived societal- and peer pressures. In this respect, (social) media and the commercial food and beverage environment may start to replace family rules and habits (learned at home), exerting a stronger influence on food and beverage choices. While young adults are experiencing these changes, it is important to consider that these are embedded in a wider societal context. For instance, indicators of lower SES have previously been identified as determinants of higher SSB intake and intake of dietary energy from sweetened beverages, overall[7,33]. The association between lower SES and higher SSB consumption has been demonstrated in high-income countries such as the UK and USA[7,34]. No equivalent study has yet investigated the role of SES influencing non-alcoholic drink preferences. In our study, we found no effect of the shared environment but this does not mean that SES is unimportant. As has been previously suggested in an elegant piece of work by Plomin (2011), growing up in a shared household may be experienced differently by each individual living in that same household. As such, the unique experience of SES may be contributing to the substantial unique environmental influence on beverage preferences seen in our study, even though SES intuitively may be considered a shared-environmental factor[35].

During adolescence, individuals likely begin to diversify the food and drinks they consume, while developing an appreciation for more complex flavours (e.g. the bitterness in alcoholic beverages, vegetables)[36]. Interestingly, variation in beverage preferences appeared to have a slightly higher environmental influence than variation in food preferences, measured in this sample at the same age[11]. For instance, the present study demonstrated a relatively low estimate for the heritability of orange juice preference (18%) compared to fruit preference (49%) (which included the liking for oranges). This suggests that liking of beverages does not simply reflect flavour but also includes factors such as texture, intensity of sweetness, effort involved in consumption etc.[37].

Perhaps more importantly, our results indicate that beverage preferences may have even greater potential for environmental modification than food preferences. This is encouraging given the concern regarding high intake of SSBs, and may reflect the potent marketing strategies of SSB manufacturers in the UK. However, other macro-level factors such as SES and the precise mechanism by which these determinants affect SSB intake and preferences will require more research if environmental intervention is to shift drink choices towards healthier options.

Strengths and limitations. Although this is the first study to examine the aetiology of a range of non-alcoholic beverage preferences, only a limited number of beverage types were included in the questionnaire. In particular, sports beverages, energy drinks, and smoothies were not included, although adolescents are by far the most frequent consumers, and the largest growing consumer group of energy and sports drinks[3,38]. In addition, the measure of liking of fruit cordial did not distinguish between sugar- or artificially-sweetened fruit cordial. However, as this was the first study to investigate preference for non-alcoholic beverage types, these broad beverage preference categories will serve as a base to inform future studies which aim to investigate beverage consumption behaviours in more detail. For instance, it would be valuable to investigate the aetiology for the liking of water, which is the beverage type consumed most frequently in this age group[39].

Preference scores were based on self-report, which is subject to bias; it is possible that adolescents underreported liking for beverages typically considered as "unhealthy". SSB consumption has previously been found to be underreported by up to 30–40% when validated against an objective blood biomarker[40], and this type of underreporting has been shown to increase with BMI[41]. However, most participants in this study had a healthy BMI, and the questionnaire data were collected anonymously.

It is possible that beverage preferences are susceptible to short-term variation, influenced by recent consumption or exposure to certain tastes or diets in the period preceding measurement[42,43]. However, the test-rest pilot study of this questionnaire indicated that beverage preference scores were reasonably reliable over a two-week period.

We only identified two twin studies that had investigated genetic and environmental influence on beverage preferences, and both examined coffee only. Further research is therefore needed to replicate our findings, and to establish if the aetiology of beverage preferences varies with developmental stage (e.g. toddlerhood, early childhood or adulthood). This is especially warranted given that energy consumed from beverages now accounts for a substantial proportion of energy intake in the modern diet, with roughly 20% of people's daily calories coming from beverages[44,45]. The sample were predominantly white British and lean, so the results need replicating in more diverse samples. Likewise, we were not able to assess whether the drink preferences of this sample were comparable to the preferences of the UK general population. Rates of underweight (10%) were higher in this subsample of TEDS, and rates of overweight (13.4%) and obesity (4.5%) were lower, relative to the UK population for this age group (underweight = 8%; overweight = 21%; obese = 16%)[46]. This may perhaps indicate that they may not entirely be representative in terms of dietary factors because they were considerably leaner. Nevertheless, participants were drawn from the TEDS study which has been shown to be reasonably representative in terms of important sociodemographic characteristics of the UK general population[47]. While these indicators do not include the primary variables under consideration, this observation suggests these findings were obtained from a sample representative of the UK's young adult (18–19 y.) population. Nevertheless, caution is warranted before the results of the study are generalised to larger, more diverse populations.

However, the large sample size and narrow age-range were strengths that allowed reliable estimates for beverage preferences to be established for an important developmental phase.

It was unknown whether the twins had already left home or were residing in a shared home at the time of data collection. The twin method relies on the assumption that MZs and DZs share their environments to a very similar extent, so if more MZs lived together than DZs (or had more frequent contact), the MZ similarity could be inflated, artificially inflating heritability. Although we were unable to rule this out, previous studies have tested the equal environments assumption and found it to be valid[48].

Lastly, focusing on beverage *preferences* rather than beverage *intake* or *consumption frequency*, has several advantages. Arguably, beverage *preferences* can be measured more accurately than actual intake, using a preference questionnaire that includes a comprehensive list of beverage types distributed to a large population sample; overcoming the high cost and inaccuracies of dietary *intake* assessments[49]. Preferences are one of the most important drivers of actual intake and have been identified as useful predictors of nutrition-related disease risk[50]. Understanding the origins of *preferences* therefore provides useful insights into the factors that need to be targeted by public health programmes to modify consumption (e.g. decreasing intake of SSBs).

Implications and future research. We established that the largest proportion of variation in beverage preferences in late adolescence is determined by factors in the individual environment. This highlights the potential for modification via public health initiatives aimed at the wider environment. At the same time, this suggests that targeting the family home environment is unlikely to modify beverage preferences among adolescents. One might look towards regulating the marketing strategies used to maximise consumption of energy-dense beverages for insights into how to reduce preferences for them. Current national and international obesity policies adopt a child-centric approach, focusing on regulating and restricting the obesogenic influences such as targeted advertisement of energy-dense food and drinks at children[51]. Preventing excessive weight gain in childhood is a public health priority as a high BMI in childhood is a predictor of overweight or obesity in adulthood[52,53]. However, adolescents' rates of obesity are rising more rapidly compared to the rest of the adult population[54], and maintenance of energy balance and healthier dietary intake in this age group also has great potential for disease prevention[55]. While children are rightly considered vulnerable members of society[56], it is short sighted to assume that once a child transitions into 'adolescence' they should be fully exposed to the unregulated commercial pressures of the permissive food and drink environment. Arguably, adolescence is an especially vulnerable developmental period during which individuals are particularly susceptible to social pressures[57,58]. The food and drink industry employ sophisticated strategies which capitalize on this vulnerability to influence adolescents' attitudes and consumption intentions of their products[59]. Our finding suggests that there might be further population health to be gained if commercial strategies that target young adults are submitted to the same level of regulation as marketing strategies that target younger children[60].

Previous interventions aimed at changing preferences for foods and beverages, rather than intake, have almost exclusively been undertaken in children perhaps because children's behaviours are considered to be more malleable[61,62]. There is also wider support for interventions targeted at children, while interventions aimed at adults can be perceived as the enforcement of 'nanny state' policies that restrict personal choice. To date, there are two studies providing experimental evidence of adult sweetness preference modification[63,64]. Both studies suggest that self-imposed reduction of sugar and sweetener intake over the course of 2 weeks[63] to 3 months[64] has the potential to reduce preferred sweetness levels in food and beverages. Future experimental studies are needed to test the feasibility of such an approach specifically aimed at the modification of sweetness intensity preferences in beverages, and if this taste preference modification can be maintained in the long-term.

Conclusion

Beverage preferences in this sample were found to be under low to moderate genetic influence, and were not influenced by aspects of the environment completely shared by adolescent twin pairs. This suggests that children's early shared family experiences may not have lasting effects on beverage preferences but further research will be required to establish whether shared environmental influences may become important again in later life. Reducing the consumption of free sugar at the population-level has in the past years moved to the top of the public health agenda, culminating in the revision of the recommended total daily energy intake from free sugars to <10% by the WHO[65]. At the same time, decreasing obesity levels remain a priority for governments worldwide. Beverages, especially SSBs and other energy-dense beverages are a major contributor to both problems, and yet population consumption levels of these types remain high. Our study emphasizes the importance of unique environmental factors in shaping non-alcoholic beverage preferences, highlighting the potential to modify preferences for unhealthy beverages by national public health programs. Specifically, for SSBs, which have the strongest evidence base for their detrimental health effects, and which can be relatively easily targeted by taxation strategies or levies, policies targeting the wider environment are promising techniques to achieve substantial population level health gains[66]. With the recent rise of national governments and jurisdictions worldwide enacting these fiscal measures, the results from the present study suggest these initiatives might be particularly effective in adolescent populations.

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