Chemosensory dysfunction as a marker of global disease:
Investigating the role of taste and/or smell signalling in obesity
and COVID-19

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Declaration

'I, Janine Maria Makaronidis 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.'

Signature:

27th June 2021
Abstract

The work in this thesis examines chemosensory dysfunction related to obesity and COVID-19, two global pandemics markedly impacting on health. Following the COVID-19 outbreak, reports emerged that loss of smell and/or taste may be caused by SARS-CoV-2. The work in Chapter 2 was undertaken before loss of smell and/or taste were recognised COVID-19 symptoms and aimed to determine the seroprevalence of SARS-CoV-2 antibodies in people with acute taste and/or smell loss, characterise the loss of chemosensory function and identify factors affecting their recovery. Overall, 78% of people with taste and/or smell loss had positive SARS-CoV-2 IgG/IgM antibodies. Female sex and altered smell/taste perceptions were identified as predictors for persistent loss of sense of taste and/or smell and long COVID. Furthermore, objective smell testing and quantitative MRI brain imaging were undertaken to investigate the underlying pathophysiology. Early results suggest ongoing neuroinflammation in people with persistent smell loss.

Obesity, a chronic disease with multiple associated co-morbidities, is associated with chemosensory dysfunction, particularly toward dietary fat. The obesity section of this thesis used different modalities, including functional taste assessment, salivary and circulating biomarkers and functional brain imaging to characterise chemosensory dysfunction in obesity. Findings from these studies demonstrated a reduced ability to taste fat in the fed state, as well as increased taste-stimulated activity in reward-related brain regions in people with obesity.

Altered adipocytokines and inflammation are postulated to underlie the increased risk of critical illness from COVID-19 in people living with obesity. In Chapter 6 inflammatory adipocytokines and metabolomic markers were measured in people with obesity before and after bariatric surgery. A substantial inter-individual variability was identified in circulating levels of these markers, which may underlie some of the susceptibility to infection-induced critical illness. Importantly, the results indicated improvement in inflammation markers following bariatric surgery, suggesting the potential for reducing obesity-associated risks.
Impact Statement

As the COVID-19 pandemic spread globally in 2020 it rapidly became clear that people living with obesity, a non-communicable disease with multiple associated co-morbidities, were more likely to develop severe COVID-19. According to the World Obesity Federation, out of 2.5 million deaths reported globally from COVID-19 by February 2021, 90% occurred in countries with a prevalence of overweight or obesity >50%. The work presented in this thesis provides novel findings on chemosensory dysfunction as manifestations of both COVID-19 and obesity, with important implications for the treatment of both diseases.

Chapter 2 presents results of a seroprevalence study, undertaken during the peak of the first COVID-19 wave when smell and taste loss were not recognised COVID-19 symptoms and it was not known if people without cough and/or fever would seroconvert to produce antibodies. We found that 78% of people from a community cohort with loss of sense of smell and/or taste had positive SARS-CoV-2 antibodies. These findings thereby improved our knowledge of the clinical course of COVID-19. Furthermore, the follow-up results from this study contributed significantly to our understanding of the resolution pattern of smell and taste loss following COVID-19 and, importantly, identified risk factors for persistent chemosensory dysfunction and long COVID. Subsequently, as shown in Chapter 3, brain imaging was performed in people with persistent and recovered anosmia, using a detailed quantitative MRI protocol, with results suggestive of ongoing neuroinflammation in people with persistent anosmia. This novel finding has implications for the treatment of patients with COVID-19 related anosmia, who may benefit from immune-modulating and anti-inflammatory therapies.

Chapter 4 provides novel information on the physiological function of GDF15, an inflammatory cytokine, which has gained interest as a potential obesity treatment due to its appetite-suppressing properties in supraphysiological doses in animal studies. The results presented provide previously undocumented evidence of a distinct GDF15 response pattern to dietary fat between plasma and saliva. Furthermore, they reveal
that GDF15 secretion in response to bariatric surgery does not follow a universal pattern, but instead depends on a person’s pre-operative levels, a novel finding. The work presented also further characterises the impaired chemosensation of dietary fat in people with obesity. fMRI results (Chapter 5) show increased taste responsiveness to both sweet and sweet/fatty taste stimuli in brain regions controlling food reward and food-related decision-making in people with obesity. Taken together these findings add to the existing knowledge on the altered eating behaviour in obesity and highlight the importance of targeting taste and reward pathways to develop effective therapeutic strategies for people living with obesity.

Finally, the work in Chapter 6 investigated the secretion pattern of adipocytokines linked to poor COVID-19 outcomes in people with obesity together with metabolomic markers, illustrating the variability of their secretion pattern, which may reflect underlying risk of infection-related critical illness. This work also illustrates how these adipocytokines and metabolic markers are altered by bariatric surgery, providing novel insights into how bariatric surgery may reduce the risk of critical illness from COVID-19 and other infections.
Contributions to the work in this thesis

The following people have contributed to work described in this thesis:

- **Chapter 2:** Dr Nyala Balogun and Miss Jessica Mok assisted with antibody testing during the FORECAST Study. Dr Cormac Magee assisted with the logistics and the running of the study. Dr Matt Lechner provided advice regarding olfactory assessments and Professor Rumana Omar oversaw the statistical analysis of the FORECAST Study.

- **Chapter 3:** Professor Claudia Gandini Wheeler-Kingshott and her team at the UCL Institute of Neurology performed image processing and calculation of biophysically meaningful features for quantitative MRI data obtained during the FORECAST Study.

- **Chapters 4 and 6:** Dr Cormac Magee and Dr Kusuma Chaiyasoot assisted with study visits and processing of blood and saliva samples in the healthy volunteer study described in Chapter 4. Dr Jason Cheung and Dr Andrea Pucci were also part of the study team carrying out study visits for the cohort of participants who had bariatric surgery. Chloe Firman helped with ELISA assays for the measurement of adipocytokines.

- **Chapter 5:** Dr Jed Wingrove carried out image pre-processing and processing for functional MRI brain images acquired as part of the TASTER Study and assisted with the statistical analysis. Dr Jed Wingrove and Dr Cormac Magee assisted with study visits during the study.
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Finally, I want to dedicate the work in this thesis to the memory of Jenny Jones, who was a source of inspiration, support and a dear friend, from my very first day at the Centre for Obesity research until she passed away in 2019.
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**List of Abbreviations**

%WL: Percentage weight loss  
a-MSH: melanocyte-stimulating hormone α  
ACE2: Angiotensin-Converting Enzyme-2  
ACTH: Adrenocorticotropic hormone  
AgRP: Agouti-related protein  
ALI: Acute lung injury  
ANOVA: Analysis of variance  
ARDS: Acute respiratory distress syndrome  
AT: Adipose tissue  
AUC: Area-under-the-curve  
BCAA: Branched-chain amino acids  
BIA: Bioelectrical impedance analysis  
BMI: Body mass index  
BOLD: Blood-oxygen-level dependent  
BPF: Bound pool fraction  
CBF: Cerebral blood flow  
CCK: Cholecystokinin  
CDC: Centres for Disease Control and Prevention  
CGM: Cortical grey matter  
CI: Confidence interval  
CNS: Central nervous system  
COVID-19: Coronavirus disease 2019  
CRP: C reactive protein  
CSF: Cerebrospinal fluid  
CT: Computerised tomography  
CTL: Cytotoxic T lymphocytes  
CVD: Cardiovascular disease  
DGM: Deep grey matter  
DHA: Docosahexaenoic acid  
DWI: Diffusion-weighted imaging
EECs: Enteroendocrine cells
ELIAA: enzyme-linked immunosorbent assay
EPI: Echo planar imaging
FA: fractional anisotropy
FFM: Fat free mass
FLAIR: Fluid-attenuated inversion recovery
fMRI: Functional magnetic resonance imaging
FSH: Follicle-stimulating hormone
GDF15: Growth differentiation factor 15
GFRAL: Glial cell line-derived neurotrophic factor family receptor α-like
GI: Gastrointestinal
GIP: gastric inhibitory polypeptide
GLP-1: Glucagon-like peptide 1
GlycA: Glycoprotein acetylation
GM: grey matter
GnRH: Gonadotropin-releasing hormone
GWAS: Genome-wide association studies
hsCRP: highly sensitive C reactive protein
ICF: Intensity curvature fraction
ICU: Intensive care unit
IFN-γ: Interferon gamma
IgG: Immunoglobulin G
IgM: Immunoglobulin M
IL-10: Interleukin 10
IL-12: Interleukin 12
IL-13: Interleukin 13
IL-17: Interleukin-17
IL-18: interleukin 18
IL-4: Interleukin 4
IL-5: Interleukin 5
IL-6 receptor
IL-6: Interleukin 6
IL-8: Interleukin 8
IMV: Invasive mechanical ventilation
iNOS: Inducible nitric oxide synthase
IR: Insulin resistance
KCH: King’s College Hospitals
kg: Kilogram
LH: Luteinising hormone
m: Metres
MC4R: Melanocortin-4 receptor
MCP-1: Macrophage chemo-attractive protein
MD: Mean diffusivity
MIC-1: Macrophage inhibitory cytokine 1
MIF-1: Macrophage inhibitory factor
MK: Mean kurtosis
mM: Millimolar
MNI: Montreal Neurological Institute
MP-PCA: Marchenko-Pastur Principal Component Analysis
MTV: Macromolecular tissue volume
MUFA: Monounsaturated fatty acids
NAFLD: Non-alcoholic fatty liver disease
NAWM: Normal appearing white matter
NICE: National Institute for Health and Care Excellence
NMR: Nuclear magnetic resonance
NODDI: Neurite orientation dispersion and density imaging
NPY: Neuropeptide Y
NRP1: Neuropilin-1 receptor
ODI: Orientation Dispersion Index
OXM: Oxyntomodulin
PCR: Polymerase chain reaction
PNS: Peripheral nervous system
POMC: Pro-opiomelanocortin
PPAR-γ2: Proliferator-activated receptor-γ2
PUFA: Polyunsaturated fatty acids
PYY: Peptide YY 36
qMRI: quantitative magnetic resonance imaging
qMT: Quantitative magnetization transfer
QSM: Quantitative susceptibility mapping
RNA: Ribonucleic acid
ROI: Regions of interest
RYGB: Roux-en-Y gastric bypass
SARS-CoV-2: Severe Acute Respiratory Syndrome Coronavirus 2
SAT: Subcutaneous adipose tissue
SD: Standard deviation
SEM: Standard error of the mean
SFA: Saturated fatty acids
SG: Sleeve gastrectomy
SPM: Specialized pro-resolving mediators
T2b: Macromolecular T
T2DM: Type 2 diabetes mellitus
TASTER: TASTE and eating behaviouR study
TE: Echo time
TGF-beta: Transforming growth factor beta
TI: Inversion time
TMPRSS2: Transmembrane serine protease 2
TNFa: Tumour necrosis factor alpha
TR: Repetition time
Tregs: Regulatory T cells
UCL: University College London
UCLH: University College London Hospitals
UHL: University Hospital Lewisham
UPSIT: University of Pennsylvania Smell Identification Test
VAT: Visceral adipose tissue
VFISO: Isotropic diffusion volume fraction
VLCD: Very low-calorie diet
WHO: World Health Organisation
WM: White matter
1. Chapter 1: Introduction

The world in 2020 was challenged by the Coronavirus disease 2019 (COVID-19) pandemic, which spread rapidly following the appearance of the Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) in late 2019. In addition, COVID-19 is interconnected with the obesity, a non-communicable disease, a pandemic which has been exponentially growing over the past decades. Obesity, a disease characterised by an accumulation of excess adipose tissue that impairs health, has been driving an unprecedented prevalence of metabolic complications such as type 2 diabetes mellitus (T2DM), cardiovascular disease, liver disease and certain types of cancer. Additionally, people living with obesity are more likely to develop critical illness with COVID-19 and have a higher COVID-19 related mortality compared to normal weight individuals.

Eating, a behaviour critical to survival, as well as for recovery from illness, relies on intact chemosensory function, through the senses of smell and taste. Chemosensory dysfunction is a common pathological feature in both COVID-19 and obesity. Understanding the physiology of smell and taste and their pathophysiology of their dysfunction could not only improve our understanding of the underlying disease processes, but could also pave the way for novel, effective treatments both for weight management as well as the sequelae of COVID-19. The body of work presented in this thesis aims to characterise chemosensory dysfunction in people with COVID-19 and in people with obesity and to gain pathophysiological insights. This chapter provides an introduction to chemosensory function, COVID-19, obesity and their respective relationships.

1.1 Chemosensory function in health and disease

1.1.1 Smell and taste: role in survival and eating

The senses of olfaction and gustation are among the most evolved senses in all species, having played a crucial role in survival. Olfaction enables surveying the chemical
composition of the outside environment and thus guides behaviour[1]. Thereby, olfaction, has played a key role throughout the evolution of species in sourcing food, eating, mating and avoiding danger[2]. The olfactory receptor cells in the nasal epithelium are of particular interest, as they are neurones. The human olfactory system can discriminate between thousands of different compounds. From the nasal olfactory epithelium, signals are conveyed to the olfactory bulb, where signal processing occurs, before information is integrated and conveyed to higher cortical centres[2]. The connection between olfaction and metabolism is well described and dates back to the work of Ivan Pavlov, who illustrated that olfactory food cues can generate endocrine responses in anticipation of a meal[3]. It is now well established that food-related smells can generate powerful metabolic responses, driving the motivation to eat[4].

Taste is a sensory modality involving the perception of food-derived chemicals in the oral cavity, as they stimulate taste receptors within taste buds[5]. From an evolutionary perspective, the sense of taste served the purpose of evaluating foods for toxicity and nutrients, thereby aiding food-related decision-making, and signalling the properties of the foods being ingested, in order to initiate metabolic responses[5]. Taste signals are integrated with olfactory signals and sensory signals about the textural properties of food within the insula in the central nervous system (CNS) to generate the perception of flavour[6]. Food palatability, defined as the positive hedonic value of the chemosensory properties of food, is a key determinant of food choice and intake, which largely stems from the perception of flavour of ingested food[7]. The palatability of energy-dense foods is among several evolutionary adaptations which conferred a survival benefit against famine, through physiological mechanisms rendering human metabolism efficient at seeking and storing energy from food[7]. Even in our modern environment, where people don’t rely on their olfaction for sourcing food and safety, taste and smell remain fundamental sensory functions, which are critical for nutrition, food selection and metabolism, for the hedonic and sensory experience of food, as well as, importantly, for a good quality of life[2].
1.1.2 Smell and taste dysfunction as markers of disease

A review of the roles of taste and smell in humans in a modern environment concluded that these relate to interpersonal communication and eating[8]. Therefore, the reported impact of the loss of smell and taste on the lives of patients who experience these, includes the inability to recognise and appreciate food, to recognise the odour of a partner or child, and the occurrence of related adverse events, such as not recognising an item is burning, or food poisoning[9,10]. Disorders of smell and taste are common. Up to 5% of people under the age of 60 experiencing olfactory impairment and a considerably higher prevalence in older adults[11]. Impaired gustatory function in the general population is also estimated to affect up to 5%[12]. Smell and taste disorders can occur as a manifestation of a wide range of disease processes[9]. Although they most commonly occur transiently during upper respiratory tract viral infections, as a result of head trauma or due to neurological disease, smell and taste loss have been associated with a number of other disease processes, including inflammatory bowel disease, certain malignancies, endocrine disorders and renal disease [9,13,14]. Furthermore, in populations of older adults, olfactory impairment has been linked to a higher mortality from neurodegenerative and cardiovascular disease[15].

Given the importance of gustatory and olfactory function for eating and metabolism, it is not surprising that metabolic conditions and extremes of body weight are accompanied by chemosensory dysfunction[16]. Both anorexia nervosa and obesity are associated with impaired olfactory and gustatory function, although to date, the exact pathophysiology of these processes is not yet understood[1]. Furthermore, impairments in smell and taste are commonly among the first symptoms noticed by patients with acute respiratory viral infections[17]. Importantly, determining the presence of chemosensory impairment is simple, non-invasive and easily applicable at population-wide level, which could lead to effective diagnostic and preventative measures. In addition, studying chemosensory dysfunction as a manifestation of systemic disease can yield crucial information about the underlying pathophysiological
processes. Therefore, investigating chemosensory function and dysfunction has potential significant benefits which are relevant from a global health perspective.
1.2 COVID-19

1.2.1 Introduction to COVID-19

SARS-CoV-2 emerged in Wuhan, China at the end of 2019. COVID-19, the acute infectious disease caused by SARS-CoV-2, spread rapidly around the globe and was declared a pandemic on 11th March 2020[18]. By the 25th June 2021, 179,513,309 cases and 3,895,661 deaths were reported worldwide[18]. SARS-CoV-2 is an enveloped single-stranded ribonucleic acid (RNA) virus and a member of the betacoronavirus subfamily[19]. SARS-CoV-2 enters human cells through the viral envelope spike (S) glycoprotein binding to the Angiotensin-Converting Enzyme-2 (ACE2) receptor[20]. SARS-CoV-2 subsequently utilises Transmembrane Serine Protease 2 (TMPRSS2) for S protein priming[21]. The ACE2 receptor is highly expressed in a multitude of human tissues, including the lungs, the upper respiratory tract, the olfactory epithelium, the oral cavity, but also along the small intestine and in adipose tissue[20]. The mean duration of the incubation period is approximately 6 days, though can last from 3-11 days, from exposure to onset of symptoms[22]. The immunological response to the virus results in neutralising SARS-CoV-2 Immunoglobulin M (IgM) and immunoglobulin G (IgG) antibodies, which bind to the SARS-CoV-2 S glycoprotein with high affinity and provide immunological memory[23]. Detectable antibodies develop approximately 1-2 weeks following the onset of symptoms[24].

1.2.2 Clinical manifestations of COVID-19

The clinical manifestations of COVID-19 are highly heterogenous with reports of COVID-19 affecting almost all body systems, as well as in terms of disease severity, ranging from asymptomatic disease to critical illness[25,26]. In the majority of patients, COVID-19 symptoms are of mild to moderate severity and can be managed in the community with supportive measures. The duration of illness and recovery from COVID-19 are also highly variable. The clinical course of symptomatic COVID-19 can be limited to 3 days of symptoms in mild cases, with means of hospitalisation of approximately 25 days in admitted patients[27,28]. The most commonly reported
COVID-19 symptoms are fever, a new persistent cough, fatigue and the loss of sense of smell and/or taste[29]. UK data suggest that 40% of people with symptomatic COVID-19 experience mild symptoms without clinical evidence of pneumonia or hypoxia[30]. A further 40% develop symptoms of moderate severity with non-severe pneumonia[30]. COVID-19 can, however, also present with a wide range of severe disease. In 15% of individuals, symptomatic COVID-19 will progress to significant disease including severe pneumonia which requires hospitalisation and in a further 5% this will result in critical illness[30].

Although COVID-19 was originally described as a primarily respiratory pathogen, the multi-system nature of the disease soon became apparent, as reports of cases affecting multiple organ systems rapidly emerged from March 2020 onward[31]. It has been suggested that the wide range and variability of pathology attributed to SARS-CoV-2 is a result of the tropism of the virus for the ACE2 receptors located on several different human tissues[32]. Figure 1.1 illustrates the range of clinical COVID-19 manifestations.
In a number of patients, recovery from COVID-19 can be prolonged, with symptoms and functional impairments lasting for several weeks or months following the acute illness. Factors associated with a prolonged recovery time include older age, admission to intensive care, pneumonia and the presence of underlying metabolic or cardiovascular disease[28,33,34]. Current data suggest that approximately two thirds of patients will return to their baseline health and functional status within 2-3 weeks from the onset of their symptoms[34].

1.2.3 Loss of the sense of smell and taste as a manifestation of COVID-19
Following original reports linking anosmia (loss of smell) and dysgeusia (loss/change of taste) to COVID-19, chemosensory symptoms are now established as characteristic symptoms of the disease. Furthermore, it has become apparent, that COVID-19 can present with anosmia and/or taste disturbance in isolation, or in the absence of respiratory symptoms and pyrexia[35,36]. In an online survey of 2428 UK patients with newly reported anosmia, 16% did not report any other symptoms. Out of the patients with other symptoms, cough and/or fever were only present in 51%[37]. Clinical studies, mainly conducted in patients attending secondary care due to COVID-19, illustrated a prevalence of anosmia and/or taste disturbance in the range of 31-85% of COVID-19 patients[38-40]. Post-viral smell loss is a well-described clinical syndrome and anosmia is a symptom that has been previously reported to occur secondary to coronavirus infections[41]. Smell loss, attributed to olfactory neuropathy was also reported in SARS-CoV[42]. SARS-CoV had also been demonstrated to invade the olfactory epithelium and the CNS via the olfactory bulb in animal models[43,44]. The pathogenicity of SARS-CoV-2 in terms of causing chemosensory dysfunction, given its 79% RNA sequence similarity to SARS-CoV, is therefore not surprising[20].

Although post-viral smell loss from other respiratory pathogens is usually a short-lived phenomenon, recovery times for smell and taste loss following SARS-CoV-2 vary considerably. Recovery times reported in the literature range from a small number of days to several months, including reports of patients who have to date not recovered their olfactory or gustatory function[45-47]. A study on SARS-CoV-2 positive patients who underwent objective olfactory and gustatory assessments during and following COVID-19, demonstrated that 70% of patients had olfactory dysfunction and 26% gustatory dysfunction during COVID-19[48]. After 28 days from resolution of the acute infection, 25% had ongoing olfactory dysfunction[48]. Given the spread of the pandemic and the fact that smell and taste loss constitute common symptoms of COVID-19, post-COVID persistent smell loss is anticipated to affect a considerable number of patients presenting to different healthcare settings. Understanding the exact pathophysiology of chemosensory dysfunction in COVID-19 and the risk factors for persistent symptoms will enable identification of those at risk and development of effective therapeutic strategies.
1.2.4 Long COVID

COVID-19 has a wide range of clinical presentations, severity and recovery period. It is now established that COVID-19 symptoms can persist, beyond the resolution of the acute viral infection[49]. Persistent symptoms are now described as a clinical syndrome, termed ‘long COVID’ or ‘chronic COVID’. Long COVID has been used to described COVID-19 related symptoms persisting for longer than 4 weeks, whereas ‘post-acute COVID-19’ has been defined as ongoing symptoms for 3-12 weeks following the infection and ‘chronic COVID-19’ as symptoms persisting beyond 12 weeks[50,51]. Data from the UK Office for National Statistics (ONS) suggest that following COVID-19, symptoms persisting longer than 5 weeks occur in approximately 1 in 5 people and symptoms lasting longer than 12 weeks in 1 in 10 people[52]. The most commonly reported long COVID symptoms are fatigue, cough, headache and loss of taste and/or smell[53]. Prevalence was reported to be higher in females compared to males and the most commonly affected age groups are 35-49 and 50-69 years. Taken together, these data suggest that in the coming months to years there will be an unprecedented demand to meet the healthcare needs of the population affected by COVID-19 in the long-term. Ongoing work aimed at delineating the exact pathophysiological drivers for these presentations and predictive tools to identify those at the greatest risk of chronic symptomatic disease will be essential in order to address the post-pandemic consequences of COVID-19, by developing and offering effective, evidence-based treatments.
1.3 Obesity: a non-communicable global pandemic

Obesity is a chronic disease that results from the excess accumulation of adipose tissue and impairs health[54]. Obesity can present in a remitting and relapsing nature and results in significant morbidity and premature mortality[54]. Obesity is classified based on Body Mass Index (BMI), which serves as a proxy for body fat, and is calculated by dividing an individual’s weight in kilograms (kg) by the square of their height in metres (m)[55]. Table 1.1 illustrates the classification of normal weight, overweight and obesity based on BMI.

Table 1.1: Classification of normal weight, overweight and obesity based on BMI

<table>
<thead>
<tr>
<th>Classification</th>
<th>BMI (kg/m²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Underweight</td>
<td>≤18.5</td>
</tr>
<tr>
<td>Normal weight</td>
<td>18.5-24.9</td>
</tr>
<tr>
<td>Overweight</td>
<td>25.0-29.9</td>
</tr>
<tr>
<td>Obesity</td>
<td></td>
</tr>
<tr>
<td>Obesity Class I</td>
<td>30-34.9</td>
</tr>
<tr>
<td>Obesity Class II</td>
<td>35-39.9</td>
</tr>
<tr>
<td>Obesity Class III</td>
<td>≥40.0</td>
</tr>
</tbody>
</table>

Classification for people of Caucasian ethnicity. Lower cut-offs apply for people of Asian origin and certain other ethnic groups[56].

According to the World Health Organisation (WHO), in 2016 over 650 million people were living with obesity world-wide, which accounts for 13% of the global adult population[55]. The consequent prevalence of obesity-associated morbidity has a detrimental impact on the individuals living with obesity, both in terms of general health and the impact upon their quality of life, but also on health-care service provision globally[57-59]. Furthermore, all-cause mortality is increased by 40% in people living with obesity compared to people of healthy weight[58]. The impact on
mortality increases further with higher BMI, with life expectancy being reduced by approximately 8-10 years in people with a BMI >40kg/m²[60].

1.3.1 Principles of appetite and body weight regulation

Before describing the pathological processes occurring in obesity, it is important to highlight the physiology of appetite regulation and the complexity of the processes involved. Eating is a behaviour essential for survival, and it is therefore not surprising that through evolutionary adaptations it has been rendered enjoyable[5]. Throughout most of the time that humans evolved, food availability has been scarce, which led to the development of powerful biological mechanisms that drive eating. Energy homeostasis is regulated by a highly complex system of physiological mechanisms, which incorporate ongoing integration of peripheral and central signals of both short- and long-term energy availability[61]. These signals are conveyed through the action of a panoply of metabolic polypeptides, originating from the gastrointestinal (GI) tract, the liver, pancreas, adipose tissue and immune system, as well as neural signals from central and peripheral networks[61]. The GI tract, which together with the adipose tissue constitute the largest endocrine systems, is the body’s first point of contact with ingested nutrients. Via the GI tract, information regarding the composition and energy content of ingested nutrients is conveyed to hypothalamic centres and brainstem regions regulating energy homeostasis. Gut hormones are secreted from enteroendocrine cells (EECs) along the entire length of the GI tract and act locally and distally, through receptors located on multiple organs and tissues, including the hypothalamus[62]. Gut hormones act in autocrine, paracrine and endocrine ways, to regulate digestive physiology, energy and glucose homeostasis and eating behaviour[62].

Within the CNS, the hypothalamus is the main centre integrating peripheral signals of energy availability into central networks. Hypothalamic nuclei containing neurones expressing neuropeptide Y (NPY) and agouti-related protein (AgRP) drive orexigenic responses, whereas melanocortin-producing neurones generate anorexigenic responses, in a mutually exclusive manner[63]. In the paraventricular nucleus of the
hypothalamus, the melanocortin-4 receptor (MC4R) promotes satiety, improves insulin sensitivity and increases energy expenditure[64]. The receptor is activated by melanocyte-stimulating hormone (a-MSH), a melanocortin, and inhibited by AgRP[64]. Loss of function MC4R mutations result in hyperphagia, severe obesity, rapid growth and impaired glucose metabolism[65]. MC4Rs have been localised on EECs and are thought to contribute to regulation of gut hormone secretion[66].

In conditions of energy-deficiency, the orexigenic hormone ghrelin is secreted predominantly from P/D1 cells in the gastric fundus[67]. Ghrelin stimulates NPY and AgRP-expressing neurones, generating a sensation of hunger and driving eating; an effect which is exacerbated by prolonged fasting[67]. In contrast, in response to nutrient ingestion, a multitude of anorectic peptides are secreted from EECs, including peptide YY 36 (PYY), glucagon-like peptide 1 (GLP-1) and oxyntomodulin (OXM), which have the opposite effect to ghrelin. These anorectic peptides activate a-MSH neurones, in turn generating a feeling of satiety and suppressing eating[68]. In addition to their direct effect on the hypothalamus, gut hormones also act on vagus nerve afferents. The vagus nerve has an innate plasticity and is capable of up- and down-regulating gut hormone receptors depending on energy availability[69,70]. Importantly, gut hormones act synergistically to regulate energy homeostasis and also interact with other signals of energy regulation[71]. For instance, GLP-1 and PYY have additive effects and gastric inhibitory polypeptide (GIP) and cholecystokinin (CCK) enhance the effects of GLP-1[72-74]. Furthermore, GLP-1, PYY and OXM have an additive effect on inducing satiety and reducing energy intake[71,75]. EECs and gut hormones additionally interact with bile acids and the intestinal microbiome[76,77].

1.3.2 Adipose tissues and adipocytokines

The adipose tissue is the largest endocrine organ in the human body and is metabolically highly active, secreting cytokines and adipokines to regulate energy homeostasis[78]. The physiological behaviour of adipose tissue is highly dynamic, responding to changes in energy availability and nutritional status[78]. Adipocytes secrete leptin, an adipokine which acts a signal of long-term energy stored in adipose
tissue[79]. Circulating leptin levels correlate with adipose tissue mass[79]. Leptin acts on the brainstem to activate pro-opiomelanocortin (POMC) neurones and simultaneously inhibits NPY/AgRP neurones[80]. In conditions of energy surplus, adipocytes expand to store lipids. Consequently, an increase in adipose tissue mass leads to higher circulating leptin levels, which in normal weight rodents results in an increase in anorectic signals and a reduced energy intake[81]. Similarly, in healthy weight humans, a rise in leptin levels following a positive energy balance results in appetite suppression, reduced energy intake and increased energy expenditure[82,83]. In contrast, in conditions of energy deficiency, circulating leptin levels drop, which results in adipose tissue releasing energy through lipolysis[81]. In addition to acting as a signal of long-term energy availability, leptin also responds to acute changes in energy availability following a meal, particularly the ingestion of dietary fat[84]. Furthermore, leptin also acts in modulating reproductive and immune function. Leptin moderates kisspeptin secretion, a neuropeptide that stimulates gonadotropin-releasing hormone (GnRH) producing neurones, thereby effecting gonadotropin secretion, a physiological process linking energy availability to reproductive function[85]. Leptin receptors are also widely expressed throughout the immune system and leptin acts as a mediator for both innate and adaptive immune responses, stimulating the secretion of multiple pro-inflammatory immune cytokines[86]. Adiponectin is an adipokine with catabolic and anti-inflammatory effects[87]. Rises in circulating adiponectin levels stimulate lipid oxidation, suppress pro-inflammatory cytokines, improve insulin sensitivity and lead to reductions in body weight[87]. Resistin has the opposite effects; it is pro-inflammatory, reduces insulin sensitivity and promotes weight gain[88]. Adipose tissue is also a major source of the pro-inflammatory cytokines, including tumour necrosis factor alpha (TNFa), interleukin 6 (IL-6), interleukin 8 (IL-8), interleukin 18 (IL-18), and the anti-inflammatory interleukin 10 (IL-10), as well as their receptors[89]. These adipose tissue-derived cytokines, as well as growth differentiation factor 15 (GDF15), an inflammation-induced cytokine, also impact upon body weight regulation[89,90]. The exact interplay between leptin, adiponectin,
resistin and the adipose tissue-mediated cytokines in the control of adipose tissue metabolism remains incompletely understood.

The distribution of adipose tissue also directly impacts upon its metabolic activity and research over the past years has highlighted the differences in adipose tissue biology. Subcutaneous adipose tissue (SAT) lies directly under the skin, whereas visceral adipose tissue (VAT) surrounds internal organs[91]. VAT is associated with a pro-inflammatory metabolic profile, increased insulin resistance and higher risks for metabolic conditions such as T2D, as well as an overall increase in mortality[92,93]. Ectopic fat, the deposition of fat outside adipose tissue, including pancreatic, epicardial and intramuscular deposits, is additionally associated with an increased cardiometabolic risk[94]. Notable sex differences also exist regarding adipose tissue distribution, with males being more prone to accumulate VAT compared to females and VAT increasing in post-menopausal females[95]. The ratio of SAT to VAT has genetic and environmental lifestyle determinants, such as nutrition, exercise and stress[91].

1.3.3 Smell, taste and food cues in energy regulation and the hedonic brain

In an environment where food availability exceeds requirements, food choices are largely driven by cognition, food presence and palatability, and by social and emotional factors[96]. The perceived reward of consuming an available food can overrule the satiety signals generated by meeting energy requirements. With the average individual having to make over 200 food-related decisions per day, food-related decision making is mostly determined by factors unrelated to homeostasis[61].

Interestingly, exposure to food cues, such as the sight or smell of food, can generate potent metabolic responses, even in the absence of energy intake, and gut hormones can influence reward-related aspects of eating behaviour[97]. Neuroimaging studies using functional Magnetic Resonance Imaging (fMRI) have provided insights into the relationship between gut hormone profiles, food cues and their reward value[98]. For instance, ghrelin levels have been shown to rise in anticipation of food consumption,
with circulating levels directly correlating with reward-centre activity in response to viewing food images[99,100]. Similarly, the smell of food can also directly affect ghrelin secretion[101]. Furthermore, ghrelin itself also increases olfactory sensitivity[102]. Likewise, administration of ghrelin was also shown to increase the pleasantness ratings of food-related smells in healthy adults[103].

Data from fMRI studies also illustrate how gut hormones can modulate activity in CNS reward centres[100,104]. For instance, following administration of intravenous PYY to healthy volunteers, mimicking the fed state, activation in the orbitofrontal cortex predicted subsequent food intake, in contrast to the hypothalamus in the fasted state, demonstrating that PYY switches the control of eating behaviour from homeostatic to reward-driven control[105]. Both PYY and GLP-1 inversely correlate with reward-responses to food-cues and their actions suppress reward responses[105,106].

The role of taste signalling in the perception of energy-dense foods as highly palatable has attracted growing scientific interest in recent years. PYY, ghrelin and GLP-1 are present in saliva and are thought to have a role in modulating taste, particularly in response to sweet and fatty stimuli[107,108]. GLP-1 is expressed in sweet and umami sensing taste buds and GLP-1 null mice have reduced taste sensitivity to sweet taste[109,110]. The ability of humans to taste fat independent of sweet, salty, bitter, sour and umami, as well as textural and olfactory cues has been well described. Interestingly, taste buds able to detect fatty acids express GLP-1, ghrelin and CCK[111,112]. At a cellular level, the fatty acid oleic acid (C18:1) has been shown to directly stimulate GLP-1 production[113]. Furthermore, oral administration of PYY to mice or over-expression of PYY in salivary glands leads to reduced food intake and decreased body weight without resulting in an increase in circulating PYY[114]. In addition, administration of an oral PYY preparation to healthy adults, resulted in increased satiety at meal onset and a 12% reduction in energy intake during an ad libitum meal. Taken together, these findings suggest that these effects are mediated through a lingual route, independent of the systemic circulation[115].
1.3.4 The pathophysiology of obesity

Weight gain occurs when energy intake chronically exceeds energy requirements. Weight gain results in a multitude of adaptive physiological changes; in gut hormone secretion profiles, adipose tissue function, as well as gustatory and olfactory function[116].

Gut hormones secretion profiles are altered with weight gain. Meal-stimulated secretion profiles of anorectic peptides, including PYY and GLP-1, are reduced in people obesity compared to people with normal weight[117]. Ghrelin secretion also becomes dysregulated, with a loss of its circadian secretion profile and pre-meal peaks combined with reduced post-prandial suppression[67,118]. Following an initial period of weight gain, circulating levels of both leptin and adiponectin rise. These elevations in leptin however don’t result in significant increases in satiety nor reductions in energy intake, suggesting that in people with obesity, leptin does not act as a regulator of body weight, or that its function is overridden by alternative mechanisms[119,120]. Therefore, treatment with leptin supplementation is ineffective at producing significant weight loss in people with obesity, except for those with a congenital leptin deficiency[121]. Following prolonged weight gain leptin signalling becomes impaired, which in turn inhibits further rises in adiponectin and levels decline[87]. Resistance to both leptin and insulin eventually develop[122]. Weight gain also results in increased oxidative stress within adipocytes, with a subsequent increase in secretion of pro-inflammatory cytokines[123]. Consequently, the metabolic sequelae of weight gain lead to appetite signals becoming disjointed from hunger and satiety.

It is important to note, that body weight is largely heritable. Studies on families, twins, and separately housed siblings, report heritability estimates of up to 70%[124]. Furthermore, genome-wide association studies (GWAS) have identified over 97 BMI-associated genetic loci[125]. Over the past decades, however, environmental and lifestyle changes, on a background genetic susceptibility, have significantly contributed toward the growing prevalence of obesity[126]. These factors have driven epigenetic changes. In addition to inter- and trans-generational effects, the epigenome is under
the constant influence from external factors, such as diet, exposure to toxins, exercise and stress, which have epigenetic consequences for weight and obesity both in childhood and adulthood[127].

1.3.5 Smell and taste in obesity

Obesity is associated with an altered eating behaviour and changes in gustatory and olfactory function are common. A high dietary fat intake over time can reduce an individual’s taste sensitivity to fatty acids and reduced gustatory sensitivity to both fat and sweet stimuli is observed in people with obesity[128,129]. This in turn may have implications for appetite and satiety, as it has been suggested that reduced fatty acid detection in the oral cavity and the GI tract leads to impaired satiety[130]. The finding that individuals hyposensitive to tasting fatty acids consume more calories compared to hypersensitive subjects following a high fat breakfast further supports this[129].

People with obesity also consistently rate energy-dense foods as more palatable compared to individuals with a healthy weight. Preference for high-fat foods has been correlated with increasing BMI and fMRI studies demonstrate higher reward responses to energy-dense foods in people with obesity[131-134]. Furthermore, in people with obesity the degree of reward centre activation in response to food cues is independent of appetite and hunger and displays a positive correlation to increasing BMI[135]. A number of fMRI studies have investigated reward responses to gustatory stimuli and have shown stronger reward responses to taste in people obesity[136-138]. Increased activity in response to tasting food has been demonstrated in both cortical and subcortical reward-related regions in people with obesity[139]. Altogether, the fMRI literature suggests that increased food-cue reactivity may be a driver for high energy intake in people with obesity. Consequently, altered metabolic and neurological responses to food may predispose individuals with obesity to further weight gain[133].

1.3.6 Current treatment strategies for obesity and the insights they offer into obesity pathophysiology
1.3.6.1 Diet and exercise

Conventional therapeutic strategies for obesity involve restricting energy intake and promoting physical activity. Although energy restriction in the short-term will result in weight loss, these reductions in weight trigger powerful biological compensatory mechanisms, aimed at defending the higher body weight, which result in weight regain in the majority people in the longer-term[140]. Sumithran et al. investigated the effects of a 10-week very low-calorie diet (VLCD) by following up a cohort of people for 52 weeks post-VLCD[141]. Significant reductions in circulating level of satiety signals including PYY, leptin and insulin, combined with increased ghrelin levels were seen at 10 weeks and persisted at 52 weeks post-VLCD[141]. Increased appetite levels, combined with persistently elevated circulating ghrelin levels, following a period of weight loss, have also been seen in people with obesity who maintained weight loss through energy restriction[142]. fMRI studies also illustrate increased reward responses to food cues following weight loss achieved through energy restriction and a recent study also suggests that activity in these reward areas of the CNS may be a predictor of longer-term outcomes following weight loss interventions[143,144]. In contrast, studies investigating the gut hormone profiles of people with obesity who achieved weight loss maintenance following energy restriction, have shown higher post-prandial PYY and GLP-1 levels[145]. Furthermore, in some individuals, exercise interventions, which have been shown to increase PYY secretion, can counteract some of the ghrelin rises seen following energy restriction and may be a successful strategy to aid weight loss maintenance in suitable patient groups[146,147]. Taken together, these findings support that weight loss maintenance with a supported follow-up plan may be achievable, however lifestyle intervention regimes are likely to be more appropriate for people with Class I or Class II obesity. Furthermore, individualising lifestyle interventions based on a person’s gut hormone profile could improve outcomes and weight maintenance in the first steps of obesity treatment algorithms.

1.3.6.2 Bariatric surgery

To date, bariatric surgery remains the most effective treatment modality for people with severe obesity, defined as people with a BMI $\geq 40\text{kg/m}^2$ or BMI $\geq 35\text{kg/m}^2$ in the presence of obesity-related co-morbidities such as T2DM[148]. Bariatric surgery leads
to a marked and sustained weight loss, resolution of co-morbidities including T2DM, hypertension, non-alcoholic fatty liver disease (NAFLD), as well a reduction in all-cause mortality[149]. In the United Kingdom (UK) the National Institute for Health and Care Excellence (NICE) recommends bariatric surgery for people with a BMI ≥40kg/m² or BMI ≥35kg/m² in the presence of obesity-related co-morbidities such as T2DM[148]. The currently most commonly performed bariatric surgical procedures are the Roux-en-Y gastric bypass (RYGB) and the sleeve gastrectomy (SG), illustrated in Figure 1.2[150]. The anatomical changes resulting from bariatric surgery engender a multitude of biological responses, which have a favourable impact on appetite and eating behaviour. RYGB results in a marked rise in meal-stimulated circulating levels of PYY, GLP-1 and other anorectic peptides, which are also seen post-SG but to a lesser extent[151]. SG in contrast, by means of removing most of the ghrelin-producing cell population, leads to a significant reduction in ghrelin levels[151,152]. These physiological changes manifest themselves as a marked change in eating behaviour, leading to a reduced energy intake, which is the main driver for weight loss.

The drastic change in eating behaviour following RYGB and SG manifests itself through reductions in hunger, increased satiety, changes in food preference away from energy-dense foods, changes in taste and smell and a reduction in the reward value of food[153-155]. Bariatric surgery shifts food preference away from energy-dense and towards low-calorie foods and leads to a marked reduction in food cravings and in the reward value of food[156,157]. Food aversions, particularly to foods high in fat and sugar are also common[155]. An increase in taste and smell acuity has also been reported, although the degree to which bariatric surgery improves taste sensitivity in addition to changing food preference and reward responses remains controversial[158-160]. Understanding the relationship between post-bariatric surgery metabolic physiology and these changes in eating behaviour, will provide valuable insights into developing novel, effective treatment approaches for obesity.
Figure 1.2: Illustrations of RYGB and SG

Illustrations of normal gastrointestinal tract (A) RYGB (B), and SG (C) from [161]. In RYGB, a small pouch, created by dividing the stomach, is anastomosed with the mid-jejunum, creating the Roux limb[162]. Thereby ingested nutrients bypass the stomach, duodenum and proximal jejunum. Pancreatic secretions and bile acids are carried via the biliopancreatic limb, and hence mix with ingested nutrients in the jejunum[162]. In SG 80-90% of the stomach is removed[163]. As a result, gastric contents pass rapidly into the duodenum. RYGB: Roux-en-Y Gastric Bypass, SG: Sleeve Gastrectomy.

1.3.6.3 Pharmacotherapy

Mimicking the beneficial effects of bariatric surgery has been a longstanding goal for pharmacological interventions to prevent and treat obesity and its complications. GLP-1 analogues already have an established role in the management of T2DM and liraglutide is now licenced in the UK for the management of obesity[164].

Administration of liraglutide, in addition to weight loss, also leads to a change in taste preference, combined with a reduction in appetite, adding to the evidence that GLP-1 may be one of the main drivers for the altered eating behaviour seen after bariatric surgery[165]. The newer, once weekly subcutaneous GLP-1 analogue semaglutide 2.4 mg, was shown to lead to an average 14.9% weight loss after 68 weeks of treatment in the phase 3 STEP-1 trial[166]. Pharmacotherapy strategies aimed at reducing ghrelin are also under development[167]. Furthermore, given the multitude of metabolic peptides secreted by the GI tract, attempts to mimic the physiological changes engendered by bariatric surgery and circumvent the adaptive biological changes that
commonly accompany weight loss through energy restriction, compounds combining multiple peptide analogues are under development and in clinical trials[168].
1.4 Obesity, low grade inflammation and immune function

1.4.1 Low grade chronic inflammation in obesity

Chronic inflammation, as evidenced by elevated pro-inflammatory cytokines and acute phase reactant C reactive protein (CRP), is a metabolic feature of obesity and a growing body of evidence is emerging suggesting it constitutes a significant risk factor for obesity-related co-morbidities including cardiovascular disease and T2DM[89]. Inflammatory cytokines, in addition to their role in immunity, also have metabolic functions in regulating insulin signalling and lipid metabolism both in healthy and pathological conditions[89,169]. Obesity results in a chronic low-grade inflammatory state, adipose tissue inflammation, increased pro-inflammatory cytokine secretion coupled with a reduction in circulating levels of anti-inflammatory cytokines, which contribute to dyslipidaemia and insulin resistance, increased risk of T2DM, cardiovascular disease, a prothrombotic state and impaired immunity[170-172]. VAT accumulation, in particular, is thought to be the driver for low grade inflammation and the secretion of pro-inflammatory cytokines[173]. The following section will review the pathophysiology of obesity-related inflammatory processes and their potential relationship to the adverse health outcomes associated with obesity.

1.4.2 Adipocytokines and their role in obesity

1.4.2.1 TNFa

TNFa is pro-inflammatory cytokine secreted in response to cell damage. In conditions of cell damage due to infection or malignancy, it is secreted by macrophages and lymphocytes[174]. TNFa is also expressed and secreted by adipocytes and a multitude of other cell types, including hepatocytes and skeletal myocytes[172]. TNFa has important roles in adipose tissue metabolism. It inhibits lipoprotein lipase, as well adipocyte differentiation, through inhibiting adipose tissue differentiation factors such as peroxisome proliferator-activated receptor-γ2 (PPAR-γ2)[175]. TNFa decreases the cellular response to insulin in adipocytes, hepatocytes and myocytes and has direct effects on cholesterol and lipoprotein metabolism in humans[176]. However, although
adipocytes express and secrete TNFa, the majority of the over-secretion of TNFa seen in obesity originates from macrophages infiltrating VAT[177].

The link between rising TNFa levels and the development of insulin resistance in obesity is now well established[175,176]. Levels of TNFa and TNFa receptor expression increase with weight gain and in individuals with obesity. TNFa levels correlate with BMI, waist circumference and waist-hip ratio [178-180]. TNFa levels also correlate both with the degree of insulin resistance and improvements in insulin sensitivity, either through weight loss or insulin-sensitising agents[181]. Elevations in TNFa, in turn, result in secretion of other pro-inflammatory cytokines, such as IL-6, and inhibit adiponectin[182].

1.4.2.2 IL-6

IL-6 is a pro-inflammatory cytokine secreted both by immune cells, including macrophages, monocytes and lymphocytes, as well as adipose tissue[183]. The IL-6 receptor (IL-6R) is expressed on multiple organs and tissues, including the hypothalamus, where it acts as a regulator of energy homeostasis[184]. IL-6 has endocrine actions, mediating its effects at sites distal to its site of secretion, via the systemic circulation[185]. IL-6 endocrine effects include stimulation of the hypothalamic-pituitary-adrenal axis during inflammatory stress and its production is directly stimulated by catecholamines[185]. In immune cells, IL-6 is secreted in response to TNFa[171]. IL-6 is one of the main cytokines in the acute-phase inflammatory response, stimulating CRP and fibrinogen production in the liver, release of white blood cells and platelets from the bone marrow and triggers haemostasis. IL-6 also has a role in glucose and lipid metabolism.

Epidemiological studies have associated elevated IL-6 with higher risk of both T2DM and obesity and chronic elevations of IL-6 is a feature of the low-grade chronic inflammation seen in obesity[171]. Chronic IL-6 elevation in obesity has been proposed as a mediator for obesity-associated complications, including the metabolic syndrome, cardiovascular disease, T2DM and may have a role in the increased risk of malignancies associated with obesity[186-188]. IL-6 and TNFa are both directly produced by adipose
tissue, and VAT in particular, suggesting a role in adipocyte metabolic function and the metabolic dysfunction seen in obesity[189]. Furthermore, the secretion of TNFα and IL-6 are interlinked. In people with obesity, levels of both TNFα and IL-6 have been shown to be elevated and show a positive correlation[169]. IL-6 also directly impairs lipoprotein lipase, contributing to the disordered fatty acid deposition seen in obesity[190]. In addition, IL-6 has been shown to inhibit insulin function, whereas inhibition of IL-6 has been shown to improve hepatic insulin sensitivity in rodent studies[171].

However, not all data link IL-6 with adverse metabolic outcomes. IL-6 can directly promote GLP-1 and GIP production and thereby stimulate insulin secretion[191,192]. It has consequently been proposed that elevations in IL-6 may form an compensatory response aimed at improving glycaemic control as insulin resistance develops[193]. In healthy humans, administration of IL-6 also leads to lipolysis, fat oxidation and a reduction in circulating triglycerides[194,195]. Furthermore, central IL-6 signalling suppresses feeding and improves energy and glucose homeostasis in obese mice[196].

Although some of these data appear contradictory, evidence suggests that the metabolic effects of IL-6 depend on the physiological conditions under which it is secreted, as well as the cell type of origin[171]. Whereas in the acute phase IL-6 levels rise up to 1000-fold above the normal range, the elevation seen in chronic inflammation in the range of 2-3 times above normal levels[171]. Administration of IL-6 to humans in high doses results in fever, hyperglycaemia and catecholamine secretion[185,197]. However, modest elevations in plasma IL-6 in healthy adults have anti-inflammatory and glucose-lowering effects[171,197]. Interestingly, the metabolically adverse effects of IL-6 on the liver are only caused by IL-6 originating from adipose tissue[198]. Overall, although the exact role of IL-6 as a metabolic regulator remains incompletely understood, its actions are context-dependent. However, over-expression of IL-6 through adipose tissue inflammation in obesity as part of a pro-inflammatory state, contributes to the development of obesity-associated complications.
1.4.2.3 IL-10

IL-10 is an anti-inflammatory cytokine with a role in limiting the immune response to pathogens during infection, thereby reducing damage to the host[199]. The relationship between obesity and IL-10 and the role of IL-10 in the pro-inflammatory state associated with obesity is yet not fully understood. Increased IL-10 levels have been measured in people with obesity compared to normal weight individuals[200]. IL-10 levels have also been shown to be lower in women with an android versus a gynoid distribution of adipose tissue[201]. Weight loss following a 12-month lifestyle intervention was shown to reduce IL-10 levels in women with obesity and elevated IL-10 levels at baseline[200]. In contrast, other studies have shown no changes in IL-10 following weight loss from energy restriction[201,202]. Elevations in IL-10 levels following weight loss through energy restriction in people with obesity have also been reported[203].

1.4.2.4 GDF15

GDF15, a metabolically active protein encoded by the macrophage inhibitory cytokine 1 (MIC-1) gene, was originally described to be a stress- and inflammation-induced cytokine, secreted in response to macrophage activation[204]. GDF15 is highly expressed in the liver, kidneys and adipose tissues and is upregulated during inflammation, tissue injury, remodelling or malignancy[204]. Increased levels are seen with ageing, smoking and intense exercise, as well as a multitude of disease processes, including malignancy, cardiovascular disease, T2DM and obesity[205,206].

A growing body of evidence supports that circulating GDF15 impacts on appetite and body weight. This area of research emerged following the observation that GDF15 was driving anorexia and weight loss in patients with cancer-associated cachexia[207]. GDF15 binds to the glial cell line-derived neurotrophic factor family receptor α–like (GFRAL) receptor and exhibits potent anorectic effects, mediated predominantly through GFRAL in the area postrema[205,208]. Data from a number of animal studies, including non-human primates, illustrate that exogenous GDF15 administration reduces energy intake, leads to weight reduction and produces taste aversions,
particularly to high-fat containing feeds[209-211]. GDF15 therefore appears to be an attractive pharmacotherapy target for obesity.

The physiological functions of GDF15 in people with obesity are however not well understood. Whereas in the presence of a malignant disease process, GDF15 levels are inversely correlated with BMI, the opposite effect is seen in obesity, where GDF15 levels are elevated[212]. In people with obesity GDF15 levels correlate with BMI, waist-to-height ratio, blood pressure, triglycerides, insulin and C-peptide levels[212]. A recent study in adults with overweight or obesity demonstrated that exercise-induced rises in GDF15 lead to a change in food preference away from high-fat foods, suggesting that the anorectic effects of GDF15 are preserved in people with obesity[213].

The GDF15 elevation in obesity is also likely a reflection of the chronic inflammatory state. Interestingly, the 4-year follow-up data from the XENical in the prevention of Diabetes in Obese subjects (XENDOS) trial demonstrate that in people with obesity without diabetes, GDF15 was an independent predictor of insulin resistance, pre-diabetes and T2DM at 4 years[214]. A study comparing cytokine expression in VAT of people with metabolically ‘healthy’ versus ‘unhealthy’ obesity, demonstrated that GDF15 was upregulated in the metabolically ‘unhealthy’ group[215]. Interestingly, despite the anorectic effects of acute rises and exogenous GDF15 administration, a study which performed Mendelian randomisation analyses based on a genetic variant in the GDF15 gene leading to a small, lifelong increase in circulating GDF15 levels, provided evidence that chronically elevated GDF15 levels lead to weight gain and an elevate BMI[216]. Taken together, these findings suggest that elevated GDF15 levels in obesity are likely a result of the chronic inflammatory state and that, rather than suppressing appetite, they are associated with weight gain and elevated BMI. The response of GDF15 secretion to weight loss is however highly heterogenous. Energy restriction in short-term fasting has been shown to result in reductions in GDF15 both in people with healthy weight and obesity[217]. A study investigating the effects of a lifestyle intervention on GDF15 showed that levels rose with weight loss in two thirds and reduced with weight loss in one third of participants[218]. More studies investigating these differences in GDF15 responses are merited.
1.4.3 Variability of adipose tissue distribution and metabolic risk

Finally, it is important to note that not all individuals with obesity develop chronic inflammation and in those who do, there is a wide spectrum in the degree of inflammatory changes observed[219]. Likewise, not all individuals with obesity are equally susceptible to the development of obesity-related co-morbidities[220]. Although the concept of ‘metabolically healthy obesity’ is disputed and in a subset of patients the absence of co-morbidities may reflect an earlier stage in the disease process, there are also significant genetic susceptibilities that contribute to the risk of developing metabolic co-morbid disease. An emerging body of evidence suggests that deposition of VAT over SAT may to a large extent determine the degree of inflammatory and metabolic changes that occur as a consequence of weight gain[221-223]. The tendency to deposit adipose tissue subcutaneously as opposed to viscerally, also varies greatly between individuals. The ability to continue to deposit fat in subcutaneous adipose tissue, instead of VAT, has been linked with a higher insulin sensitivity, independent of BMI[224]. Adipocyte size has also been identified as a risk factor for metabolic disease in obesity[225]. Both sex and ethnicity have also been demonstrated to affect adipose tissue distribution and the associated risk of obesity-associated metabolic diseases, suggesting genetic factors may be key determinants of the size and distribution of adipose tissue cells in obesity[226]. Although the processes determining adipose tissue distribution remain incompletely understood, GWAS have identified a number of loci containing genes that regulate adipose tissue distribution[227]. In addition, some evidence also suggests that adipose tissue distribution is, at least to a certain extent, modifiable through exercise interventions and some pharmacological agents, although data in this field remains very limited[228].

1.4.4 Immune cells in obesity and chronic adipose tissue inflammation

In conditions of a positive energy balance, adipocytes in VAT accumulate large quantities of fatty acids, leading to adipocyte expansion initially, which eventually results in hyperplasia and hypertrophy. The large amount of intracellular fatty acids results in increased fatty acid oxidative processes, eventually resulting in oxidative
stress and nitric oxide accumulation. Consequently, immune cells, predominantly macrophages and cytotoxic T and Th1 lymphocytes, are recruited to the adipose tissue, which in addition to the rising leptin levels, also lead to excess secretion of pro-inflammatory cytokines including TNFa, IL-2 and IL-6, combined with a reduction in anti-inflammatory cytokine IL-10 and adiponectin[229]. Rising levels of TNFa, which is secreted by both macrophages and adipocytes, directly impair insulin action, by inhibiting insulin signalling at its receptor[172].

Immune cells involved in innate immunity, including macrophages, mast cells, neutrophils and dendritic cells have been shown to contribute to the pathogenesis of insulin resistance[230-232]. Macrophages are the most abundant immune cells in adipose tissue in obesity and are the main cell group driving adipose tissue inflammation, by secreting pro-inflammatory cytokines[229]. Adipokines, including adiponectin and leptin, modulate macrophage activation. Macrophages are often classified into M1 and M2 subtypes. M1, (‘classically activated’), macrophages, are pro-inflammatory and secrete inflammatory cytokines including IL-1β, IL-6, TNFa and inducible nitric oxide synthase (iNOS)[233]. M2, (‘alternatively activated’) macrophages, are anti-inflammatory and have a role in resolving inflammation and in tissue repair[233]. M2 macrophages secrete IL-10, thereby inhibiting IL-1β and iNOS[233]. Although it is now accepted that this classification into two types may represent an oversimplification, the ratio of pro- and anti-inflammatory macrophages is altered in obesity, with the majority of the macrophage population in obesity developing a pro-inflammatory phenotype[170,234]. Female sex has, however, been demonstrated to have a protective role against the pathophysiology of this pro-inflammatory phenotype, which is encountered more commonly in males[235].

Adipocyte hyperplasia results in increased expression of macrophage chemo-attractive protein (MCP-1) and macrophage inhibitory factor (MIF-1), attracting macrophages and driving ongoing immune cell adipose tissue invasion[236]. IL-1β, IL-6 and TNFa all stimulate MCP-1 secretion, which is predominantly expressed in visceral compared to subcutaneous adipose tissue[237]. Increased VAT MCP-1 expression precedes insulin resistance, suggesting that these processes are at least contributory to the
development of insulin resistance[236]. Furthermore, adipose tissue hyperplasia and remodelling also lead to structural and functional alterations within the adipocytes themselves. For instance, adipocytes located furthest away from supplying blood vessels develop hypoxia and consequently necrosis, which attracts phagocytic cells aiming to remove necrotic cells. Within the adipocytes these processes result in reticular stress and abnormalities in protein folding, which can trigger apoptosis. Cell death also attracts macrophages into the adipose tissue through activation of pattern-recognition receptors, which respond to chemokines released by dying cells. Infiltrating adipose tissue macrophages surround necrotic adipocytes, forming crown-like structures, which are a distinctive feature of chronic adipose tissue inflammation[238].

In addition to macrophages and the innate immune system, there is also substantial evidence linking the adaptive immune system both to obesity-related inflammation, as well as impaired immune responses to infection[236]. Lymphocytes comprise up to 10% of non-adipocyte cells within adipose tissue in humans and are present in the crown-like structures seen in adipose tissue in obesity[239]. Severe obesity has been associated with higher numbers circulating CD4+ T cells in peripheral blood, compared to lean individuals[240]. Table 1.2 summarises the role and findings about different T cell subtypes in obesity.
Table 1.2: T cell subtype physiology and their role in obesity

<table>
<thead>
<tr>
<th>T cell</th>
<th>Trigger</th>
<th>Cytokine</th>
<th>Role[236]</th>
<th>In obesity</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD4+ T cells</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Th1 cells</td>
<td>IL-12 and IFN-g</td>
<td>IFN-g</td>
<td>Pro-inflammatory: secrete IFN-g and TNFα and promote macrophage migration and differentiation into M1 subtype</td>
<td>Causal role in IR development. Cell populations increase in obesity, especially in VAT[241,242]. Cell numbers correlate with hsCRP[243].</td>
</tr>
<tr>
<td>Th17 cells</td>
<td>IL-17</td>
<td>IL-17</td>
<td>Pro-inflammatory: secrete IL-17 (Inhibits insulin-stimulated glucose uptake by skeletal muscle and hepatocytes and impairs adipocyte differentiation[244]).</td>
<td>Increased Th17 numbers in VAT[243]. IL-17 correlates with IR.</td>
</tr>
<tr>
<td>Th2 cells</td>
<td>IL-4</td>
<td>IL-4 and IL-13</td>
<td>Secrete anti-inflammatory cytokines IL-10, IL-1, IL-4, IL-5 and IL-13.</td>
<td>Protective against IR. Cell numbers inversely correlate with IR and hsCRP[243].</td>
</tr>
<tr>
<td>Tregs</td>
<td>IL-2</td>
<td>IL-10</td>
<td>Anti-inflammatory: secrete IL-10 and TGF-beta. Suppress macrophages.</td>
<td>Tregs cells counts decreased in peripheral blood and VAT[243,245].</td>
</tr>
</tbody>
</table>

CD8+ T cells

<table>
<thead>
<tr>
<th>T cell</th>
<th>Trigger</th>
<th>Cytokine</th>
<th>Role[236]</th>
<th>In obesity</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD8+</td>
<td>IFN-g</td>
<td>To kill infected/foreign cells.</td>
<td>Increased cells in VAT. Drive AT inflammation and IR[246].</td>
<td></td>
</tr>
</tbody>
</table>

TNFα: Tumour Necrosis Factor alpha, IFN-g: Interferon gamma, AT: Adipose Tissue, hsCRP: highly sensitive C reactive protein, IR: Insulin resistance, VAT: Visceral Adipose Tissue, IL-4: Interleukin 4, IL-5: Interleukin 5, IL-10: Interleukin 10, IL-12: Interleukin 12, IL-13: Interleukin 13, IL-17: Interleukin-17, TGF-beta: Transforming Growth Factor beta.

Similar to T cells, B cell populations also infiltrate VAT in obesity[247]. B cells, in addition to presenting antigens and generating antibodies also produce a range of cytokines, particularly IL-6 and TNFα, which are oversecreted in the presence of insulin resistance and T2DM[248]. In summary, the immune cell type profile within human adipose tissue is significantly altered in people with obesity, with a pro-inflammatory cell population recruited into adipose tissues and driving the chronic inflammatory state seen in obesity, as well as insulin resistance.
1.4.5 Implications for immunity in people with obesity

Obesity has consequences for immune function, not only in terms of increased susceptibility to infection, but also with regard to increased duration of infections, reduced inflammatory responses and immune cell activation during infection and worse clinical outcomes in both bacterial and viral infections, compared to individuals with normal weight[249].

Immune responses to ribonucleic acid (RNA) viruses rely on type 1 inflammatory responses by Th1 cells, combined with functional anti-inflammatory responses by regulatory T cells (Tregs) for immune response resolution[250]. Impairments in the function of these processes may attenuate the immune response to viral infections. Tregs populations, which primarily resolve immune cell-mediated inflammation following infection have also been shown to be increased in obesity. However, animal studies have shown Tregs in obesity to lead to reduced production of anti-inflammatory cytokine IL-10, an effect thought to be mediated by insulin[251]. Obesity is a known independent risk factor for morbidity and mortality from the influenza virus[252]. Studies in infection models (H1N1) have also demonstrated that Tregs in obese mice were 40% less suppressive compared to those in lean mice[253]. Combined with increased cytotoxic CD8+ cells, these findings suggest that the increased inflammatory responses in obesity may contribute to impaired immunity leading to more severe disease in viral infections[253]. The following section of this chapter will provide an overview of the links between obesity and the increased disease severity and mortality in COVID-19.
1.5 COVID-19 and obesity: One pandemic driving another

1.5.1 COVID-19 in people with obesity

The world in 2020 was facing the consequences of two pandemics: COVID-19, a communicable disease with detrimental effects on people’s lives and livelihoods globally; and obesity, a non-communicable chronic disease, driving the rise of multiple complications with a significant impact on morbidity and mortality. The COVID-19 pandemic has highlighted the vulnerability of people living with obesity to infectious diseases. Obesity increases both the risk of contracting an infection, as well as the disease severity of infectious diseases, with implications for prognosis and increased mortality[254]. This increased risk of contracting an infection was also seen with COVID-19; a pooled analysis of studies with published data on COVID-19 in people with obesity, demonstrated that the odds of a person with obesity to be positive for COVID-19 were 46% higher (OR = 1.46; 95% CI, 1.30–1.65; p <0.0001) compared to an individual without obesity[250].

The data about COVID-19 disease severity in people with overweight and obesity also illustrate significantly worse prognoses for people living with obesity. According to the Intensive Care National Audit & Research Centre (ICNARC) report on COVID-19 in critical care from 26th March 2021, 79.7% of patients admitted to critical care in the UK from September 2020 onwards suffered from either overweight or obesity[255]. A report by the Centres for Disease Control and Prevention (CDC) including data from almost 150,000 patients with COVID-19 in the US, highlights obesity as a risk factor for hospitalisation and death, especially in adults aged under 65 years[256]. A total of 50.8% of people included in the analysis had obesity. These data illustrated a non-linear, J-shaped relationship between BMI and the respective risks of hospitalisation, ICU admission and death. People with BMI between 23.7-25.9kg/m² had the lowest risk for all three outcomes, with risks rising exponentially with increasing BMI. Overall, data from a series of studies suggest that hospitalised patients with COVID-19 and obesity are younger than normal weight hospitalised patients with COVID-19, more likely to require mechanical ventilation and more likely to die from COVID-19[250,256-260].
Furthermore, the World Obesity Federation carried out a global epidemiological analysis on COVID-19 mortality, which suggests that out of the 2.5 million deaths reported globally due to COVID-19 by the end of February 2021, 90% occurred in countries where the prevalence of overweight or obesity is greater than 50%[261]. Their findings suggest that overweight is a global predictor for developing healthcare requirements due to COVID-19, independent of a country’s relative wealth, the age structure of its population or its reporting capacity[261]. Table 1.3 illustrates the relationship between overweight prevalence and COVID-19 mortality globally. Taken together, these findings suggest that a lower prevalence of overweight and obesity could have prevented a proportion of deaths from COVID-19, but also highlight the urgency to understand the pathophysiological drivers underlying this association and improve both the therapeutic and preventative strategies for people living with overweight and obesity.

Table 1.3: Adult overweight prevalence and COVID-19 mortality globally

<table>
<thead>
<tr>
<th>Prevalence of overweight in adults (% of population)</th>
<th>COVID-19 mortality per 100,000 population</th>
<th>Number of countries</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;30%</td>
<td>6.6</td>
<td>38</td>
</tr>
<tr>
<td>30-40%</td>
<td>0.5</td>
<td>19</td>
</tr>
<tr>
<td>40-50%</td>
<td>3.2</td>
<td>13</td>
</tr>
<tr>
<td>50-60%</td>
<td>67.9</td>
<td>55</td>
</tr>
<tr>
<td>&gt;60%</td>
<td>65.6</td>
<td>39</td>
</tr>
</tbody>
</table>

Table adapted from[261]

1.5.2 Physiological drivers for increased COVID-19 susceptibility and severity in people with obesity

There are multiple hypotheses aimed at explaining the association between obesity and increased hospitalisation, critical illness and mortality from COVID-19 in people living with obesity. Figure 3 summarises these proposed mechanisms. People living with obesity often experience impairments in physical functioning and Yates et al. demonstrated a link between self-reported slow walking, a marker for physical functioning, and increased risk for severe COVID-19[262]. Irrespective of the
physiological links between obesity and severe COVID-19, obesity can pose challenges for the delivery of critical care in COVID-19, in view of factors such as the requirement for increased ventilatory pressures, prolonged weaning from mechanical ventilation and difficulties with prone positioning[263]. Studies aiming to investigate the link between obesity and poor outcomes in COVID-19 have highlighted metabolic dysfunction, chronic low-grade inflammation and impaired immune functions are the main pathophysiological drivers for increased disease severity[250].

**Figure 1.3: Summary of the impact of physiological consequences of obesity on COVID-19 outcomes.**

Figure summarising the clinical manifestations and physiological consequences of obesity contributing to an increased risk of severe illness from COVID-19. (Figure from [250]). SPM: Specialized Pro-resolving Mediators, CTL: Cytotoxic T lymphocytes, IL-6: Interleukin 6, CRP: C Reactive Protein, GM-CSF: Granulocyte Macrophage Colony Stimulating Factor, TNFa: Tumour Necrosis Factor alpha, IFN-γ: Interferon gamma, CVD: Cardiovascular Disease, ICU: Intensive Care Unit; IMV: Invasive Mechanical Ventilation, ARDS: Acute Respiratory Distress Syndrome, ALI: Acute Lung Injury.

One of the hallmarks of severe disease in COVID-19, is the ability of the SARS-CoV-2 virus to generate a cytokine storm. Cytokines implicated in COVID-19 related cytokine storm include IL-2, IL-6, IL-12, IL-1β and TNFa, the production and secretion of which is known to be dysregulated in people with obesity and associated with obesity-related chronic inflammation[29,264]. Both IL-6 and TNFa expression have been associated with worse outcomes in COVID-19[265,266]. IL-6, however, has been suggested as the
most predictive cytokine, in terms of risk of critical illness, as people with IL-6 levels greater than 80pg/mL were found to be at a 22-fold increased risk of respiratory failure due to COVID-19[267]. In addition, high cytokine and chemokine expression in monocyte and leukocyte populations from samples obtained from patients with COVID-19, suggest innate immune system–mediated tissue injury as a link between obesity and critical illness from COVID-19[249]. Furthermore, GDF15, which is known to have predictive value in terms of clinical outcomes in sepsis and cardiac disease, has also been shown to have prognostic value in COVID-19[268]. Myhre et al., in a study of 123 hospitalised COVID-19 patients in Norway, demonstrated that higher GDF15 levels were associated with hypoxia and worse clinical outcomes. In their study, GDF15 had a higher prognostic value for worse clinical outcomes, compared to IL-6 and CRP[268]. A total of 27% of the patients in the study were people with obesity. A number of smaller studies have also shown associations between elevations in GDF15 levels with worse clinical outcomes and mortality in COVID-19[269,270]. Although these studies have not explicitly looked at the association GDF15 in COVID-19 mortality in people with obesity, baseline GDF15 levels are increased in people with obesity and GDF15 is known to reduce clearance of human rhinovirus and increase the risk of virus-induced lung inflammation in animal studies[271]. Taken together these findings suggest a potential role for GDF15 in critical illness from COVID-19 in people with obesity.

The association between impaired immune function in people with obesity secondary to low grade chronic inflammation driven by excess adipose tissue has also been proposed as a contributing factor for the increased disease severity often seen in people with obesity and COVID-19. Watanabe et al. investigated the relationship between abdominal VAT on COVID-19 severity in hospital patients undergoing computerised tomography (CT) imaging and demonstrated that VAT was significantly higher in people requiring admission to intensive care and associated with worse clinical outcomes[272].

Furthermore, the ACE2 receptor, which is expressed in adipose tissue, is upregulated in people with obesity[273]. This allows for increased viral replication within adipose tissues, increased viral shedding and amplification of cytokine production with the
potential of worsening COVID-19 disease severity[274]. However, to date, no study has demonstrated an increased viral load in the respiratory tract in people with obesity and COVID-19 pneumonia[275]. In contrast, adiponectin levels may directly impact upon the risk of severe COVID-19 pneumonia in people with obesity. Adiponectin is known to have a protective effect on lung capillaries and in a study by Kearns et al., low adiponectin levels were detected in patients who developed respiratory failure secondary to COVID-19 pneumonia[276]. The same study included a control cohort of patients with respiratory failure due to different pathogens, where the same relationship with low adiponectin levels was not observed, suggesting a specific role for adiposity-mediated inflammatory changes in the pathogenesis of severe COVID-19[276].

Obesity-related co-morbidities, particularly cardiovascular disease, T2DM and respiratory disease have a detrimental impact on COVID-19 related outcomes[277,278]. Respiratory dysfunction associated with obesity, such as hypoventilation or obstructive sleep apnoea, can affect outcomes in patients requiring respiratory support due to COVID-19[250]. Furthermore, hyperglycaemia and suboptimal glycaemic control in people with T2DM have been linked to an increased mortality[279]. T2DM, in particular has been associated with increased mortality due to COVID-19 at a population level[280]. Hyperglycaemia is an established driver of pro-inflammatory cytokine secretion and macrophage activation and in patients with T2DM higher glucose levels have been associated with worse clinical outcomes[279]. Zhu et al., illustrated the impact of glycaemic control on COVID-19 mortality in a population of T2DM patients[279]. The overall COVID-19 mortality in T2DM patients was 7.8%; however in the subgroup of patients with good glucose control mortality was 1.1%, which rose to 11% in those with poor glucose control[279].

COVID-19 does not universally result in critical illness in people with obesity. Evidence suggests that people with obesity and an unfavourable metabolic profile are at an excessively high risk of critical illness and mortality due to COVID-19. It is also relevant to note that, whereas obesity is more common in women and the prevalence of COVID-19 is equal in males and females, the majority of patients with severe COVID-19 are
male. In line with the fact that female sex has a protective role against meta-
inflammation and dysregulated inflammation is more common in males, sex-related
differences in the immune response to SARS-CoV-2 may lead to an increased risk of
developing a cytokine storm in males with COVID-19[281]. Androgens in males can also
lead to increased levels of IL-10 during the immune response to viral antigens, which
may attenuate the inflammatory response to the viral infection[282]. Understanding
the risk associated with low grade chronic inflammation and developing methods to
stratify this will be crucial to preventing severe illness in the context of infection in
people with obesity and targeting the underlying processes by offering effective
treatments to those at risk.

1.5.3 Consequences of the COVID-19 pandemic for people with obesity

The impact of the COVID-19 pandemic globally will be significant and long-lasting. For
people living with obesity there have been additional major implications associated
both with the disease itself, as well as the consequences of the wider measures taken
to curb the spread of the pandemic. For people living with obesity who survived COVID-
19, especially more severe disease, there are numerous implications at a personal and
society level. A study investigating weight outcomes following hospital admission in
COVID-19 survivors at 3 months, showed a significant increase in BMI compared to
their BMI at admission in people with overweight and obesity, compared to individuals
of normal weight who remained weight-stable[283]. Furthermore, initial weight loss in
the first month from admission was identified as a predictor of weight gain at 3 months.
These findings illustrate the metabolic vulnerability of people with obesity to further
weight gain as a result of COVID-19. Physical deconditioning, either as a direct result of
severe illness, the reduction physical activity due to lockdown measures, or both, is
also likely to have detrimental consequences for physical functioning, body
composition and the risk of obesity-related co-morbidities[284]. In addition, although
the exact prevalence of obesity among long COVID cohorts has not yet been quantified,
people with long COVID have been shown to have a significantly higher BMI compared
to people with COVID-19 whose symptoms resolved following the acute phase[285].
Furthermore, the fact that clinical services for people living with obesity were suspended through the peaks of the pandemic, combined with the reduction in physical activity during periods where ‘stay at home’ orders were in place are likely to have majorly impacted upon the health of people living with obesity[286]. Data from a consultation with people living with obesity also highlighted an increase in weight-related stigma surrounding the fact that people with obesity have an increased COVID-19 related mortality, combined with major concerns about mental health[287]. A meta-analysis on the impact of the March-May 2020 pandemic on body weights across population studies has shown significant weight-gain trends in adults and adolescents[288]. The financial consequences of the COVID-19 pandemic also have direct impacts upon eating behaviours, with energy-dense foods being cheaper and more accessible[289,290]. Although data on the effect of the COVID-19 pandemic and the lockdown measures on the prevalence of obesity and associated co-morbidities are only just beginning to emerge and the extent of implications is not yet known, the potential consequences for the obesity pandemic are alarming. This highlights an urgency to improve both the accessibility of weight management services and effective treatment approaches. In addition, amid concerns for potential future waves of COVID-19, but also for potential future outbreaks of viral disease, the increased mortality in people with obesity from COVID-19 has highlighted the urgency to understand the physiological drivers for this increased susceptibility, in order to be develop approaches to mitigate obesity-associated mortality when exposed to outbreaks of viral disease in the future.

1.6 Conclusion
The COVID-19 pandemic has highlighted the need to maximise health outcomes for people living with obesity. People living with obesity are at an increased risk of developing severe disease and mortality from COVID-19. Furthermore, weight management will become a priority in the long-term care of people with obesity, who have been directly or indirectly affected by the COVID-19 pandemic. Chemosensory dysfunction is a common pathological feature in both COVID-19 and obesity. The work presented in this thesis was aimed at improving our understanding of the underlying pathophysiology of chemosensory dysfunction in obesity and COVID-19, in order to
Contribute to increasing our understanding of the underlying disease processes, which is essential in order to develop novel, effective treatments for obesity and COVID-19. Chapters 2 and 3 focus on chemosensory dysfunction in COVID-19. The studies presented in Chapter 2 investigates the relationship between acute smell and/or taste loss in COVID-19. Chapter 3 presents some preliminary results from MRI imaging in order to elucidate the pathophysiology of COVID-19 related smell loss. Chapters 4 and 5 are focused on taste and fat signalling in obesity and how these are altered through bariatric surgery. Finally, the studies presented in Chapter 6 aimed to investigate the link between circulating fatty acid and adipocytokine profiles in obesity, which have been associated with poor outcomes in COVID-19 and investigate if their secretion profiles can be improved through bariatric surgery.
1.7 Chapter 1 References


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2. Chapter 2: Finding Out if COVID-19 Infection can be pREdicted by ChAnge in Smell and/or Taste (FORECAST) Study

2.1 Introduction

Although COVID-19 was originally described as a primarily respiratory disease, reports on COVID-19 presenting with anosmia and taste disturbance, often in the absence of cough and fever, rapidly emerged from March 2020. On March 21 2020, ENT UK issued a press release stating there is evidence suggesting that a considerable number of patients with COVID-19 infection have developed loss of smell and/or taste[1]. This publication additionally highlighted the fact that loss of smell and/or taste may occur as a result of COVID-19 in the absence of cough and fever, which at the time were the only symptoms triggering self-isolation and testing in the UK[1]. These observations rapidly led to a global concern about the potential public health implications of loss of smell and taste as COVID-19 presentations, which at the time, did not meet guidelines for isolation and testing in most countries[2]. A series of focused research efforts subsequently aimed at characterising the chemosensory symptomatology of COVID-19[3]. Subsequently, data from the COVID symptom study, which utilised an app-based approach to collect self-reported data on COVID-19 symptoms and testing, published by Menni et al., including responses from over 2 million participants from the UK and United States, demonstrated the predictive value of smell and taste loss in the diagnosis of COVID-19[4]. A total of 65% out of the 18,401 participants who reported a positive COVID-19 polymerase chain reaction (PCR) test result, experienced a loss of taste or smell. According to a predictive model developed by the authors, loss of smell and taste was the strongest predictor of COVID-19, with a stronger predictive value than more established symptoms, such as fever and a new, persistent cough[4]. The app however used a single question which enquired about both the loss of smell and taste; hence the results of this study did not allow discrimination between the predictive value of smell loss versus taste loss. The work presented in the first part of this chapter therefore aimed to investigate acute loss of smell and taste loss, separately
and in combination, as presentations of COVID-19 and to determine their relationship to SARS-CoV-2 IgG/IgM status.

The Finding Out if COVID-19 Infection can be pREdicted by ChAnges in Smell and/or Taste (FORECAST) Study was designed in order to quantify the seroprevalence of SARS-CoV-2 specific antibodies in a cohort of patients with an acute loss in their sense of smell and/or taste during the COVID-19 outbreak in London, UK and to determine whether a new loss/reduction in the sense of smell and/or taste were indicative of COVID-19, irrespective of the presence of cough and or fever.

By May 2020, loss of smell and taste were beginning to be incorporated into testing and self-isolation guidelines for COVID-19. Knowledge about the clinical course and predictive value of these symptoms, as well as risk factors for ongoing chemosensory impairment, however, remains limited. Most studies on COVID-related anosmia stem from patients already attending a healthcare service due to COVID-19 or from self-reported data. Furthermore, although several pathophysiological hypotheses have been proposed, the exact mechanisms through which SARS-CoV-2 impairs chemosensory function also remain incompletely understood. Elucidating the underlying pathophysiology will however be essential, in order to offer more specific and effective treatments to people whose chemosensory symptoms persist beyond the acute phase of the infection.

Recovery times for taste and smell loss following COVID-19 are reported to range from a few days to several months[5-7]. This observation that a subset of patients with COVID-related anosmia have not recovered their sense of smell following their infection, raised concerns about prolonged smell loss as a manifestation of long-COVID and illustrates the need to identify predictors for persistent smell loss[8]. Given the large number of patients affected by COVID-related smell loss and long-COVID, being able to identify those at the highest risk will be essential in order to be able to offer timely treatment.
Therefore, we followed up the FORECAST cohort over a 4-6-week period, aiming to identify risk factors for persistent loss of smell and/or taste. Finally, objective olfactory testing was performed on a subset of participants, aiming to see how well the symptoms reported in the FORECAST study correlated with objectively assessed olfactory function.

2.2 Methods

The FORECAST study was carried out between 23rd April 2020 and 20th July 2020. The study, an observational cross-sectional general public cohort study, aimed to investigate if an acute loss in the sense of smell and/or taste are indicators of COVID-19. It also aimed to describe the clinical course and factors affecting the resolution of these symptoms.

The study was planned in late March 2020, after reports emerged that loss of the sense of smell and/or taste may be caused by COVID-19 and was conducted at a time when loss of smell and taste were not recognised as symptoms. Recruitment was timed to capture people who developed symptoms during the peak of the first COVID-19 outbreak in London and recruitment took place from the 23rd April 2020 until the 14th May 2020.

Invitations to participate in the study were sent out via the patient text messaging system of 4 participating General Practice Surgeries in North London. The following message was sent out to registered patients over the age of 18: “Has your sense of smell or taste reduced in the last month? If yes and you’d like to be part of a COVID-19 research study, go to: [link]”. The link directed potential participants to an online platform, hosted by Dendrite Clinical Systems (https://www.e-dendrite.com). The platform included information on the study and the participant information sheet, as well as contact details for the research team. Once participants decided they would like to take part, the website directed them to an eligibility check and the study consent form. Participation in the study was voluntary and written informed consent was obtained electronically.
The inclusion criteria for the study were age $\geq 18$ years, an acute loss or reduction in the sense of smell and/or taste in the preceding 4 weeks, proficiency in written and spoken English and access to video calling. Participants with a pre-existing loss or impairment of their sense of smell or taste of longer than 4 weeks’ duration were excluded. Enrolled participants completed an online questionnaire (Baseline questionnaire, see appendix 1), which captured the following data:

- Demographic data: sex, age, ethnicity
- Smoking status
- Previous COVID-19 testing
- Smell and taste symptoms:
  - Presence of smell and/or taste loss
  - Degree of chemosensory impairment (complete vs partial)
  - Distorted chemosensory function
  - Date of onset
- Other symptoms of COVID-19

After completing the online questionnaire, participants were booked in for a telemedicine consultation via video call and were sent a point-of-care testing kit. Antibody testing was chosen as a means to capture positive cases without the limitations of the time window of PCR testing and in order to test participants without face to face contact. Participants who had previously been tested using a PCR test, as per the national COVID-19 testing guidelines at the time, were included in the study and tested irrespective of the PCR result. The tests used in the study were a lateral flow immunoassay (Wuhan UNscience Biotechnology Co., Ltd. COVID-19 Antibody IgM/IgG)[9]. The antibody tests underwent validation testing, using serum samples collected from COVID-19 patients from multiple hospitals and Chinese CDC laboratories. The tests were done separately at each site. A total of 1585 cases were tested: 421 (positive) clinically confirmed (including PCR) COVID-19 patients and 1164 controls. The test results showed that the product has a relative sensitivity of 98.81%
(95% CI: 97.25%, 99.61%) and a relative specificity of 98.02% (95% CI: 97.05%, 98.74%)[9].

During the video consultations, participants were asked to describe their symptoms and were subsequently supervised in obtaining a finger-prick blood sample and performing the antibody test. The results were discussed with participants at the end of the consultation and photographs of the test cassettes were obtained. The photographs were reviewed independently by a second healthcare professional in order to validate the results, before these were entered into the study database. In case of result ambiguity, the antibody test was repeated. Testing was carried out between 24th April and 22nd May 2020.

**Figure 2.1: SARS-CoV-2 antibody test cassettes**

![Image of antibody test cassettes](image)

Figure illustrating SARS-CoV-2 antibody test cassettes for each of the possible results: A. IgM positive, B. IgG positive, C. IgM and IgG positive, D: Negative.

At 4 weeks from the date participants completed their baseline questionnaire, a link to a second follow-up questionnaire was sent to them. The follow-up questionnaire included questions about the resolution of their smell and taste symptoms, as well as the other COVID-19 symptoms they reported on their baseline questionnaire and a question about whether they were admitted to hospital (Follow-up questionnaire, Appendix 2). Participants who had not completed the follow-up questionnaire were sent reminders containing the link to the questionnaire after 72 hours and 7 days. The
link to the questionnaire and the online platform remained open for a further 4-week period. Follow-up data were collected between 22\textsuperscript{nd} May and 20\textsuperscript{th} July.

Following completion of the follow-up period, in order to assess how self-reported smell function correlates with an objective evaluation of olfactory function, a subsample of participants who completed the follow-up questionnaire were recruited for objective olfactory assessment. Participants who consented to being contacted again following completion of the follow-up questionnaire were approached and were sent a separate PIS for this part of the study. Written informed consent was obtained electronically. In order to undertake an objective olfactory assessment remotely, the British version of the University of Pennsylvania Smell Identification Test (UPSIT) (MediSense, www.smelltest.eu), a 40-item ‘scratch and sniff’ test, which is validated to be self-administered, was used\cite{10}. The UPSIT generates a score out of 40, which following adjustment for age and sex, classifies olfactory function into normosmia, mild microsmia, moderate microsmia, severe microsmia and total anosmia\cite{10}.

**Figure 2.2: University of Pennsylvania Smell Identification Test (UPSIT)**

Enrolled participants were contacted and in view of the time elapsed between the completion of the follow-up questionnaire and the UPSIT testing, participants were asked to rate their smell function at the time of the test. UPSIT test kits were sent to participants and they were asked to complete the test. The correct smells for the items tested were not revealed to participants and pictures of their answer cards were obtained (Figure 2.2B). The smell test was subsequently scored, and the results communicated to the participants and their general practitioner. UPSIT testing was carried out between 21st July and 26th October 2020.

The FORECAST study received ethical approval by the Queens Square Research Ethics Committee (IRAS Project ID 282668, ClinicalTrials.gov: NCT04377815) and was conducted in line with the declaration of Helsinki and the standards of Good Clinical Practice.

2.2.1 Sample size calculation

A sample size calculation was performed in order to determine the recruitment target for the study. At the time of study design, the King’s College London/ZOE symptoms reporting application was the only source of information regarding the frequency of reported symptoms due to COVID-19[11]. A total of 1.5 million users were registered on the app on 24th March 2020 and 26% reported at least one symptom through the app between 24-29 March 2020. 1,702 people reported having been tested using PCR during that time frame and 579 tested positive. These data available from the app showed that 59% of SARS-CoV-2 positive people reported loss of smell or taste (the app at the time included one question for both taste and smell). It was therefore estimated that approximately 63% (342/544) of people with taste or smell loss were SARS-CoV-2 positive. A precision-based sample size calculation was performed. Using a conservative estimate of 50% (95% confidence interval 45-55%) for people who would test positive for SARS-CoV-2 IgM/IgG antibodies among the population with acute smell and/or taste loss recruited into the study, the sample size required was calculated at 385 participants[12]. A 15% attrition rate was included, increasing the sample size to 453 participants. The recruitment target was set to 500 participants to allow for a larger attrition and to increase accuracy. The recruitment target was
eventually exceeded, in view of a higher than anticipated recruitment rate, in order to further improve accuracy and improve the quality of the data acquired on the clinical course and resolution of smell and/or taste loss due to COVID-19.

### 2.2.2 Statistical analysis

Data were analysed using GraphPad Prism version 8 (https://www.graphpad.com/scientific-software/prism/), STATA version 15 (https://www.stata-uk.com) and SPSS version 26 (https://www.ibm.com/uk-en/products/spss-statistics). Descriptive statistics were carried out. Means plus standard deviation (SD) were calculated for continuous variables and numbers (n, with percentages [%]) for categorical variables. Continuous data were assessed for normality using a D’Agostino-Pearson omnibus normality test and parametric or non-parametric tests were performed as appropriate. Categorical data were analysed using chi-squared tests. The significance level for multiple comparisons were adjusted using a Bonferroni correction. Logistic regression analyses were performed. A Spearman rank correlation analysis was undertaken to correlate participants’ perceived smell function with their objective olfactory function, as per their UPSIT result.

### 2.3 Results

#### 2.3.1 Study population

The four participating primary care centres in North London (The Hampstead Group Practice, the Northern Medical Centre, the James Wigg Practice and the Queen’s Crescent Practice) sent out staggered text messages to their registered patient population, in waves of 5000-6000 messages at a time. A total of 33,650 text messages were sent out during the recruitment period and 650 participants completed the registration process and consented to take part in the study. Of these, 60 were not eligible and were subsequently excluded. Out of the 590 eligible participants 567 (96.1%) underwent testing for SARS-CoV-2. Data from the 567 participants tested are included in the baseline analysis.
A total of 467 participants responded to the follow-up questionnaire, yielding a follow-up rate of 82.1%. This cohort includes two further participants who were sent test kits but had not made contact with the study team to arrange their tests by the time the baseline statistical analysis was performed, who were retrospectively added to the cohort, increasing the number of participants to 569. A CONSORT diagram illustrating the study recruitment period can be seen in Figure 2.3. Results for the baseline analysis were published in PLOS Medicine in October 2020[2] and results from the follow-up analysis in BMC infectious diseases in February 2021[13].

Figure 2.3: FORECAST Study CONSORT diagram

Consort diagram illustrating participant flow through the recruitment process, from text message invitations, eligibility screening, baseline questionnaire completion, testing through to follow-up
questionnaire completion[14]. Follow up cohort includes 2 further participants who completed antibody testing after the baseline analysis was performed. Figures presented as percentages (%) with total numbers (n). Age presented as a mean with standard deviation.

2.3.2 Acute loss of the sense of smell and/or taste in the study population

Out of the 590 participants who enrolled in the study and filled out the baseline symptom questionnaire, 90.0% (n=531) reported a loss in their sense of smell and 89.8% (n=530) a loss in their sense of taste. 10.0% (n=59) reported no smell loss and 10.1% (n=60) no taste loss. Combined loss of smell and taste was reported by 80.0% (n=472). Out of participants with smell loss, 69.9% (n=371) described this as a complete loss of their sense of smell and 30.1% (n=160) as a partial loss. Loss of the sense of taste was described as complete by 47.4% (n=251) and as partial by 52.6% (n=279).

2.3.3 The seroprevalence of SARS-CoV-2 IgG/IgM antibodies in a community cohort with acute loss of their sense of smell and/or taste

Out of the 590 participants enrolled in the study, 567 (96.1%) completed antibody testing at the time of primary analysis. A total of 77.4% (n=439) of participants who underwent antibody testing were positive for SARS-CoV-2 IgG/IgM. Out of the antibody tests, 303 tests were positive for SARS-CoV-2 IgG, 14 for SARS-CoV-2 IgM and 122 for IgG and IgM. One further participant was included on the basis of a positive SARS-CoV-2 PCR result (total positive rate 77.6%). The total number of SARS-CoV-2 positive participants was 77.6% (n=440). Fifteen participants had been tested positive for SARS-CoV-2 on a PCR test in line with the national testing strategy prior to enrolling in the study and all 15 (100%) has a positive IgG/IgM antibody test. Importantly, 52.1% (n=229) of SARS-CoV-2 positive participants did not report a cough and 39.8% (n=175) had neither a fever nor a cough. The participants with positive and negative SARS-CoV-2 antibodies respectively, were comparable in terms of their age, sex and ethnicity. Table 2.1 illustrates the demographics of the study population by SARS-CoV-2 antibody status.
Table 2.1: Study group demographics by SARS-CoV-2 antibody test result

<table>
<thead>
<tr>
<th></th>
<th>SARS-CoV-2 antibody positive (n=440)</th>
<th>SARS-CoV-2 antibody negative (n=127)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Sex (%)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>70.7% (n=311)</td>
<td>63.8% (n=81)</td>
<td>0.223</td>
</tr>
<tr>
<td>Male</td>
<td>28.9% (n=127)</td>
<td>36.2% (n=46)</td>
<td></td>
</tr>
<tr>
<td>Other</td>
<td>0.5% (n=2)</td>
<td>0.0% (n=0)</td>
<td></td>
</tr>
<tr>
<td><strong>Age (mean, SD, years)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>39.1 (±8.9)</td>
<td>40.7 (±9.8)</td>
<td>0.250</td>
</tr>
<tr>
<td><strong>Ethnicity (n, %, with data)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>White</td>
<td>79.5% (n=350)</td>
<td>78.7% (n=100)</td>
<td>0.754</td>
</tr>
<tr>
<td>Asian</td>
<td>6.4% (n=28)</td>
<td>5.5% (n=7)</td>
<td></td>
</tr>
<tr>
<td>Black</td>
<td>2.7% (n=12)</td>
<td>1.6% (n=2)</td>
<td></td>
</tr>
<tr>
<td>Mixed</td>
<td>5.9% (n=26)</td>
<td>7.1% (n=9)</td>
<td></td>
</tr>
<tr>
<td>Other</td>
<td>4.8% (n=21)</td>
<td>4.7% (n=6)</td>
<td></td>
</tr>
<tr>
<td>Not disclosed</td>
<td>0.7% (n=3)</td>
<td>2.4% (n=3)</td>
<td></td>
</tr>
<tr>
<td><strong>Smoking (%)</strong></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Current smokers</td>
<td>8.4% (n=37)</td>
<td>12.6% (n=16)</td>
<td>0.153</td>
</tr>
<tr>
<td>Non-/Ex-smokers</td>
<td>91.6% (n=403)</td>
<td>87.4% (n=111)</td>
<td></td>
</tr>
</tbody>
</table>

SARS-CoV-2: Severe Acute Respiratory Syndrome Coronavirus 2, SD: Standard deviation

**2.3.4 Frequency of reported loss of smell and/or taste in participants with positive and negative SARS-CoV-2 antibodies**

Out of 440 participants with a positive SARS-CoV-2 result, 93.4% (n=411) reported a loss of smell. This was reported as a complete loss of smell in 69.8% (n=307) and a partial loss of smell in 23.6% (n=104). A total of 6.6% (n=29) reported no change in their sense of smell. Out of 127 participants with a negative SARS-CoV-2 antibody test, 78.7% (n=100) reported a loss of smell, which was reported as a complete loss of smell in 39.4% (n=50) and a partial loss of smell in 39.4% (n=50), whereas 21.2% (n=27) did not report a loss of smell. Loss of smell, including both a complete and a partial loss of...
smell, were reported with a significantly higher frequency in participants who had a positive compared to a negative test for SARS-CoV-2 antibodies (p<0.001) (Table 2.2).

Similarly, 90.2% (n=397) of participants with positive SARS-CoV-2 antibodies reported a loss of taste, reported as complete by 47.5% (n=209) and partial by 42.7% (n=188). A total of 9.8% (n=43) reported no loss of taste. In participants without SARS-CoV-2 antibodies loss of taste was reported by 89.0% (n=113). This was described as a complete loss of the sense of taste by 26.8% (n=34) and partial loss by 62.2% (n=79). A total of 11.0% (n=14) reported no change in their sense of taste. Although the frequency of reported taste changes was comparable between the two groups (p=0.738), participants with positive SARS-CoV-2 antibodies reported a complete loss of their sense of taste more frequently than participants without antibodies (p<0.001), who more commonly reported a partial loss of taste (p<0.001) (Figure 2.4).

Loss of both the sense of taste and smell were reported by 83.6% (n=368) of participants with positive SARS-CoV-2 antibodies, compared to 67.7% (n=86) of participants with a negative antibody test (p<0.001).
Figure 2.4: Frequency of reported loss of smell (A) and loss of taste (B) in participants with and without SARS-CoV-2 antibodies

A. Reported frequency of loss of smell in participants with and without SARS-CoV-2 antibodies

- Out of 440 participants with positive SARS-CoV-2 antibodies, 93.4% (n=411) reported a loss of smell. This was a complete loss of smell in 69.8% (n=307) and a partial loss of smell in 23.6% (n=104). 6.6% (n=29) reported no change in their sense of smell.
- Out of 127 participants with a negative SARS-CoV-2 antibodies, 78.7% (n=100) reported a loss of smell, which was a complete loss in 39.4% (n=50) and a partial loss of smell in 39.4% (n=50), whereas 21.2% (n=27) reported no loss of smell. Loss of smell, including both a complete and a partial loss of smell, were significantly more frequently reported participants with positive compared to a negative SARS-CoV-2 antibodies (p<0.001).

B. Reported frequency of loss of taste in participants with and without SARS-CoV-2 antibodies

- 90.2% (n=397) of participants with positive SARS-CoV-2 antibodies reported a loss of taste (complete loss of taste in 47.5%, n=209 and partial in 42.7%, n=188). 9.8% (n=43) reported no loss of taste.
- In participants without SARS-CoV-2 antibodies loss of taste was reported by 89.0% (n=113) (complete loss of the sense of taste by 26.8%, n=34 and partial loss by 62.2%, n=79). 11.0% (n=14) reported no loss of taste. The frequency of reported taste changes in was comparable between the two groups, p=0.738). Participants with positive SARS-CoV-2 antibodies reported a complete loss of their sense of taste more frequently than participants without antibodies (p<0.001).

SARS-CoV-2: Severe Acute Respiratory Syndrome Coronavirus 2, SD: Standard deviation.
Table 2.2: Reported loss of sense of smell and/or taste in participants with positive and negative SARS-CoV-2 antibodies

<table>
<thead>
<tr>
<th></th>
<th>SARS-CoV-2 antibody positive (n=440)</th>
<th>SARS-CoV-2 antibody negative (n=127)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Loss of the sense of smell</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(complete and partial)</td>
<td>93.4% (n=411)</td>
<td>78.7% (n=100)</td>
<td>p&lt;0.001</td>
</tr>
<tr>
<td>Complete loss of smell</td>
<td>69.8% (n=307)</td>
<td>39.4% (n=50)</td>
<td>p&lt;0.001</td>
</tr>
<tr>
<td>Partial loss of smell</td>
<td>23.6% (n=104)</td>
<td>39.4% (n=50)</td>
<td></td>
</tr>
<tr>
<td>Loss of the sense of taste</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(complete and partial)</td>
<td>90.2% (n=397)</td>
<td>89.0% (n=113)</td>
<td>p=0.738</td>
</tr>
<tr>
<td>Complete loss of taste</td>
<td>47.5% (n=209)</td>
<td>26.8% (n=34)</td>
<td>p&lt;0.001</td>
</tr>
<tr>
<td>Partial loss of taste</td>
<td>42.7% (n=188)</td>
<td>62.2% (n=79)</td>
<td></td>
</tr>
<tr>
<td>Combined loss of sense of smell and taste</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(complete and partial)</td>
<td>83.6% (n=368)</td>
<td>67.7% (n=86)</td>
<td>P&lt;0.001</td>
</tr>
<tr>
<td>Complete loss of both smell and taste</td>
<td>50.5% (n=186)</td>
<td>20.5% (n=26)</td>
<td>P&lt;0.001</td>
</tr>
<tr>
<td>Complete loss of smell, partial loss of taste</td>
<td>25.6% (n=94)</td>
<td>15.7% (n=20)</td>
<td></td>
</tr>
<tr>
<td>Partial loss of smell, complete loss of taste</td>
<td>4.6% (n=17)</td>
<td>3.9% (n=5)</td>
<td>p=0.642</td>
</tr>
<tr>
<td>Partial loss of both smell and taste</td>
<td>19.3% (n=71)</td>
<td>27.6% (n=35)</td>
<td>p&lt;0.001</td>
</tr>
</tbody>
</table>

SARS-CoV-2: Severe Acute Respiratory Syndrome Coronavirus 2.

Parosmia, a distorted perception of the sense of smell, was reported by 30.0% (n=133) of participants with a positive SARS-CoV-2 antibody test result and 21.3% (n=27) of participants with a negative antibody test (p=0.245). Dysgeusia, a distorted perception of the sense of taste, was reported by 44.7% (n=198) versus 42.9% (n=54) in participants with positive and negative antibody tests respectively (p=0.856). A total of 21.4% (n=95) of participants with SARS-CoV-2 antibodies, compared to 24.6% (n=31)
of those with negative antibodies, reported experiencing sensations of taste while not eating or drinking (phantom taste) (p=0.374). Extracts of descriptive comments provided by participants with positive SARS-CoV-2 antibodies in their questionnaire comment spaces are illustrated in Table 2.3.

**Table 2.3: Extracts of participants’ comments describing their loss of smell and/or taste**

<table>
<thead>
<tr>
<th>Examples of descriptions of participants’ loss of their sense of smell</th>
</tr>
</thead>
<tbody>
<tr>
<td>“Sense of smell vanished, couldn't smell anything from garlic to bleach to aromatherapy oils.”</td>
</tr>
<tr>
<td>“Phantom scent of burning/ammonia/petrol/chemicals”</td>
</tr>
<tr>
<td>“I could not smell anything at all, from dirty nappies to bleach, although this was not accompanied by nasal or sinus congestion”</td>
</tr>
<tr>
<td>“I couldn't smell anything. The neighbour’s apartment caught on fire one night and if it wasn't for my flatmate (or the fire brigade later) I wouldn’t have realised.”</td>
</tr>
<tr>
<td>“It was as if the nerves had fried. My nose was not blocked, I just suddenly was unable to smell anything.”</td>
</tr>
<tr>
<td>“Zero smell...not even strong things like frying garlic and perfumes. It was unlike when I have had similar experiences with colds because my nasal passages were not blocked and I could breathe normally.”</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Examples of descriptions of participants’ loss of their sense of taste</th>
</tr>
</thead>
<tbody>
<tr>
<td>“I could not taste even the spiciest of foods or sweetest. I tried different chillies too but nothing had a taste.”</td>
</tr>
<tr>
<td>“I could only taste very basic things like bitterness or sweetness, couldn’t pick up any aromas or flavours in food or drinks.”</td>
</tr>
<tr>
<td>“I could taste absolutely nothing. I tested various food and drink but absolutely nothing. I did not have a cold.”</td>
</tr>
<tr>
<td>“I couldn’t taste chillies or any food. Drinks were just liquid.”</td>
</tr>
<tr>
<td>“I could taste absolutely nothing. I tested various food and drink but absolutely nothing.”</td>
</tr>
<tr>
<td>“It was a very sudden onset, one day I woke up and could not taste anything at all”</td>
</tr>
</tbody>
</table>
2.3.5 Additional COVID-19 symptoms reported

The most common additional symptoms reported in both the SARS-CoV-2 antibody positive and negative groups were headaches (64.3% vs 63.0%), muscle and/or joint pains (60.2% vs 52.0%) and loss of appetite (57.3% vs 56.0%). No statistically significant differences were observed in the frequency of reported additional symptoms between participants with positive and negative SARS-CoV-2 antibodies. Twenty nine (6.6%) participants with SARS-CoV-2 antibodies and 10 (7.9%) with a negative antibody test reported no additional symptoms. Importantly, 52.1% (n=229) of participants with SARS-CoV-2 antibodies did not report a cough and 39.8% (n=175) had neither a fever nor a cough. Cough and fever were reported by 25.7% of participants. Although these differences did not reach statistical significance, sore throat, abdominal pain and diarrhoea were more common in participants with a negative SARS-CoV-2 antibody test. Table 2.4 illustrates a breakdown of reported additional symptoms in participants with positive and negative SARS-CoV-2 antibodies respectively.
Table 2.4: Additional COVID-19 symptoms reported

<table>
<thead>
<tr>
<th>Other symptoms (%, with data)</th>
<th>SARS-CoV-2 antibody positive (n=440)</th>
<th>SARS-CoV-2 antibody negative (n=127)</th>
<th>p value¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>Headache</td>
<td>64.3% (n=283)</td>
<td>63.0% (n=80)</td>
<td>0.784</td>
</tr>
<tr>
<td>Muscle and/or joint pain</td>
<td>60.2% (n=265)</td>
<td>52.0% (n=66)</td>
<td>0.096</td>
</tr>
<tr>
<td>Loss of appetite</td>
<td>57.3% (n=252)</td>
<td>56.0% (n=71)</td>
<td>0.784</td>
</tr>
<tr>
<td>Cough</td>
<td>47.7% (n=210)</td>
<td>48.0% (n=61)</td>
<td>0.952</td>
</tr>
<tr>
<td>Sore throat</td>
<td>40.9% (n=180)</td>
<td>52.0% (n=66)</td>
<td>0.027</td>
</tr>
<tr>
<td>Chest pain and/or tightness</td>
<td>37.7% (n=166)</td>
<td>31.5% (n=40)</td>
<td>0.198</td>
</tr>
<tr>
<td>Fever</td>
<td>37.7% (n=167)</td>
<td>25.2% (n=32)</td>
<td>0.080</td>
</tr>
<tr>
<td>Shortness of breath</td>
<td>37.1% (n=163)</td>
<td>33.9% (n=43)</td>
<td>0.511</td>
</tr>
<tr>
<td>Confusion, disorientation and/or drowsiness</td>
<td>32.3% (n=142)</td>
<td>33.9% (n=43)</td>
<td>0.737</td>
</tr>
<tr>
<td>Diarrhoea</td>
<td>25.6% (n=117)</td>
<td>34.7% (n=44)</td>
<td>0.076</td>
</tr>
<tr>
<td>Hoarse voice</td>
<td>17.3% (n=76)</td>
<td>21.3% (n=27)</td>
<td>0.305</td>
</tr>
<tr>
<td>Abdominal pain</td>
<td>15.0% (n=66)</td>
<td>24.4% (n=31)</td>
<td>0.013</td>
</tr>
<tr>
<td>Vomiting</td>
<td>3.6% (n=16)</td>
<td>1.6% (n=2)</td>
<td>0.243</td>
</tr>
</tbody>
</table>

¹ No significant differences were detected between the two groups, in any parameter reported in this table. The significance level for other symptoms was adjusted for multiple comparisons to p=0.004 through a Bonferroni correction. SARS-CoV-2: Severe Acute Respiratory Syndrome Coronavirus 2.

2.3.6 Loss of smell as a predictor of positive SARS-CoV-2 antibodies in a community-based population with acute loss of their sense of smell and/or taste

A logistic regression analysis was performed in order to explore the relative importance of loss of smell and loss of taste, both as isolated symptoms and in combination, as symptoms indicative of a COVID-19 infection, assessed by the presence of SARS-CoV-2 antibodies in this study population with an acute loss of smell and/or taste. The regression model compared an isolated loss of smell and combined loss of smell and taste to an isolated loss of taste. Results of the logistic regression analysis are presented in Table 2.5.
Table 2.5: Logistic regression exploring the seroprevalence of SARS-CoV-2 antibodies in people with loss in the sense of smell in isolation, loss in the sense of taste in isolation and a loss both in the sense of smell and taste in combination

<table>
<thead>
<tr>
<th></th>
<th>Odds ratio (95% CI) (unadjusted)</th>
<th>p value</th>
<th>Odds ratio (95% CI) (adjusted)*</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Isolated loss of taste (baseline)</td>
<td>1.00</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Isolated loss of smell</td>
<td>2.86 (95% CI: 1.27 to 6.36)</td>
<td>&lt;0.001</td>
<td>2.72 (95% CI: 1.21 to 6.14)</td>
<td>0.016</td>
</tr>
<tr>
<td>Combined loss of smell and taste</td>
<td>3.98 (95% CI: 2.24 to 7.08)</td>
<td>&lt;0.001</td>
<td>4.11 (95% CI: 2.29 to 7.37)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Constant</td>
<td>1.07 (95% CI: 0.64 to 1.81)</td>
<td>0.789</td>
<td>2.91 (0.75 to 11.35)</td>
<td>0.123</td>
</tr>
</tbody>
</table>

*Adjusted for sex, age, ethnicity and smoking status (CI: confidence interval).

Participants with an isolated loss of smell were nearly 3-times more likely than participants with isolated taste loss to have a positive test for SARS-CoV-2 antibodies (OR 2.86, 95% CI 1.37 to 6.36; p<0.001) and participants with a combined loss of smell and taste were 4-times more likely to have SARS-CoV-2 antibodies (OR 3.98, 95% CI 2.24 to 7.08; p<0.001). The significance of these findings remained unchanged after adjusting for sex, age, ethnicity and smoking status.

2.3.7 Resolution of smell and/or taste symptoms in participants tested for SARS-CoV-2 IgG/IgM antibodies at 4-6 week follow-up

After a 4-6 week follow-up period, 467 participants completed the study’s follow-up questionnaire, yielding a follow-up rate of 82.1%. The follow-up cohort included 381 participants with positive SARS-CoV-2 antibodies and 86 with negative antibodies. The participants from the two groups remained comparable in terms of their age, sex, ethnicity and smoking status (Table 2.6).
Table 2.6: Demographics of participants followed up after 4-6 weeks with positive and negative SARS-CoV-2 antibodies

<table>
<thead>
<tr>
<th>Demographics</th>
<th>SARS-CoV-2 IgG/IgM positive (n=381)</th>
<th>SARS-CoV-2 IgG/IgM negative (n=86)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Gender</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>70.9% (n=270)</td>
<td>66.3% (n=57)</td>
<td>0.382</td>
</tr>
<tr>
<td>Male</td>
<td>28.8% (n=110)</td>
<td>33.7% (n=29)</td>
<td></td>
</tr>
<tr>
<td>Other</td>
<td>0.3% (n=1)</td>
<td>0</td>
<td>0.450</td>
</tr>
<tr>
<td><strong>Age (years)</strong></td>
<td>39.67±12.12</td>
<td>40.25±12.33</td>
<td>0.689</td>
</tr>
<tr>
<td>**Ethnicity *</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>White</td>
<td>83.7% (n=319)</td>
<td>81.4% (n=70)</td>
<td>0.600</td>
</tr>
<tr>
<td>Mixed/Multiple Ethnicities</td>
<td>5.5% (n=21)</td>
<td>4.7% (n=4)</td>
<td>0.749</td>
</tr>
<tr>
<td>Asian/Asian British</td>
<td>5% (n=19)</td>
<td>4.7% (n=4)</td>
<td>0.897</td>
</tr>
<tr>
<td>Black/African/Caribbean/Black British</td>
<td>1.6% (n=6)</td>
<td>2.3% (n=2)</td>
<td>0.628</td>
</tr>
<tr>
<td>‘Other’</td>
<td>3.7% (n=14)</td>
<td>3.5% (n=3)</td>
<td>0.933</td>
</tr>
<tr>
<td><strong>Smoking status</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Current/ Ex-smoker</td>
<td>42% (n=160)</td>
<td>44.2% (n=38)</td>
<td>0.710</td>
</tr>
<tr>
<td>Never smoked</td>
<td>58% (n=221)</td>
<td>55.8% (n=48)</td>
<td></td>
</tr>
</tbody>
</table>

*5 participants opted not to disclose their ethnicity.

SARS-CoV-2: Severe Acute Respiratory Syndrome Coronavirus 2, SD: Standard deviation
Out of 467 participants in the follow-up cohort, 57.7% (n=206) of those with positive SARS-CoV-2 antibodies reported full resolution of their smell loss, compared to 72.1% (n=49) of participants with a negative antibody test (p=0.027). Out of the participants with positive SARS-CoV-2 antibodies, 38.4% (n=137) reported partial and 3.9% (n=14) reported no resolution of their smell loss at the time of follow-up. Out of participants with negative SARS-CoV-2 antibodies 25.0% (n=17) reported partial and 2.9% (n=2) no resolution of their smell loss (Figure 2.5).

Figure 2.5: Smell (A) and taste loss resolution (B) in participants with and without SARS-CoV-2 IgG/IgM antibodies at 4-6 week follow-up

With regard to the loss of taste, this was reported to have resolved by 66.2% (n=227) of participants with SARS-CoV-2 antibodies and 80.3% (n=61) of participants with negative antibodies (p=0.017). A total of 31.2% (n=107) of participants with positive antibodies reported partial and 2.6% (n=9) no resolution of their taste symptoms by the time of follow-up. Out of participants with negative SARS-CoV-2 antibodies, 19.7% (n=15) reported partial resolution of their taste loss and no participants reported ‘no resolution’ of their loss of taste. For subsequent analyses participants with partial and no resolution of their smell and taste loss respectively, were grouped together in order
to enable comparison between participants who achieved full resolution versus those who had an ongoing smell and/or taste impairment at the time of follow-up (Table 2.7).

Table 2.7: Smell and/or taste loss resolution in participant with positive and negative SARS-CoV-2 IgG/IgM antibodies

<table>
<thead>
<tr>
<th>Pattern of resolution</th>
<th>SARS-CoV-2 IgG/IgM positive</th>
<th>SARS-CoV-2 IgG/IgM negative</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Smell loss</td>
<td>Total (n=357)</td>
<td>Total (n=68)</td>
<td></td>
</tr>
<tr>
<td>No/partial resolution</td>
<td>42.3% (n=151)</td>
<td>27.9% (n=19)</td>
<td>0.027</td>
</tr>
<tr>
<td>Full resolution</td>
<td>57.7% (n=206)</td>
<td>72.1% (n=49)</td>
<td></td>
</tr>
<tr>
<td>Taste loss</td>
<td>Total (n=343)</td>
<td>Total (n=76)</td>
<td></td>
</tr>
<tr>
<td>No/partial resolution</td>
<td>33.8% (n=116)</td>
<td>19.7% (n=15)</td>
<td>0.017</td>
</tr>
<tr>
<td>Full resolution</td>
<td>66.2% (n=227)</td>
<td>(80.3%) (n=61)</td>
<td></td>
</tr>
<tr>
<td>Combined smell and taste loss</td>
<td>Total (n=261)</td>
<td>Total (n=54)</td>
<td></td>
</tr>
<tr>
<td>No/partial resolution</td>
<td>36.0% (n=94)</td>
<td>20.4% (n=11)</td>
<td>0.026</td>
</tr>
<tr>
<td>Full resolution</td>
<td>64.0% (n=167)</td>
<td>79.6% (n=43)</td>
<td></td>
</tr>
</tbody>
</table>

SARS-CoV-2: Severe Acute Respiratory Syndrome Coronavirus 2.

In participants with positive SARS-CoV-2 antibodies who reported full resolution of their smell loss, a full recovery of the sense of smell was reported to have occurred within one week in 11.7%, within 1-2 weeks in 26%, within 2-4 weeks in 26.5% and within >4 weeks in 35.8%. Table 2.8 illustrates the temporal resolution pattern of smell and taste loss in patient with SARS-CoV-2 IgG/IgM antibodies.
Table 2.8: Resolution pattern of smell and taste loss in patients with SARS-CoV-2 IgG/IgM antibodies who reported full resolution

<table>
<thead>
<tr>
<th>Time to full resolution</th>
<th>&lt; 7 days</th>
<th>1-2 weeks</th>
<th>2-4 weeks</th>
<th>4-12 weeks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Loss of smell (n=196)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(n=23)</td>
<td>11.7%</td>
<td>26.0%</td>
<td>26%</td>
<td>35.7%</td>
</tr>
<tr>
<td>Loss of taste (n=187)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(n=20)</td>
<td>10.7%</td>
<td>24.6%</td>
<td>30.5%</td>
<td>34.2%</td>
</tr>
</tbody>
</table>

SARS-CoV-2: Severe Acute Respiratory Syndrome Coronavirus 2.

2.3.8 Factors affecting resolution of smell and/or taste loss following the acute phase in participants with SARS-CoV-2 IgG/IgM antibodies

a. The effect of smell loss pattern

A full recovery of their sense of smell was reported by 54.5% of participants who experienced a complete loss of smell compared to 67.4% in participants who reported a partial loss of their sense of smell (54.5% vs 67.4%, p=0.032).

b. The effect of parosmia

Out of participants who reported parosmia at the time of their original symptoms, 41.4% experienced a full recovery of their sense of smell compared to 65% of participants who did not experience parosmia (41.4% vs. 65%, p<0.001).

c. The effect of taste loss pattern

Full recovery of the sense of taste was reported with comparable frequency in participant who reported complete vs. partial loss of their sense of taste (64.7% vs. 67.9%, p=0.525).

d. The effect of dysgeusia

Out of participants who experienced dysgeusia at time of their original symptoms, a significantly lower proportion reported full recovery of their sense...
of taste, compared to participants who did not experience dysgeusia (60.7% vs. 71.4%, p=0.036).

e. The effect of phantom taste experiences

Participants who experienced taste sensations in the absence of eating or drinking reported lower resolution rates compared to participants who did not (51.2% vs 71.0%, p=0.001).

f. The effect of sex

Compared to participants of female sex, a significantly higher percentage of male participant experienced full resolution of their loss of smell (72.8% vs. 51.4%, p<0.001), their loss of taste (80.8% vs. 60.1%, p<0.001) and combined loss of smell and taste (69.6% vs. 45.1%, p<0.001) (Table 2.9).

Table 2.9: Resolution of loss of smell, loss of taste and combined loss of smell and taste in female vs male participants with SARS-CoV-2 IgG/IgM at 4-6 weeks follow-up

<table>
<thead>
<tr>
<th></th>
<th>Female</th>
<th>Male</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Smell loss resolution</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Full resolution</td>
<td>51.4% (n=130)</td>
<td>72.8% (n=75)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>No/partial resolution</td>
<td>48.6% (n=123)</td>
<td>27.1% (n=28)</td>
<td></td>
</tr>
<tr>
<td><strong>Taste loss resolution</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Full resolution</td>
<td>60.1% (n=146)</td>
<td>80.8% (n=80)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>No/partial resolution</td>
<td>39.9% (n=97)</td>
<td>19.2% (n=19)</td>
<td></td>
</tr>
<tr>
<td><strong>Combined smell and taste loss resolution</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Full resolution</td>
<td>45.1% (n=102)</td>
<td>69.6% (n=64)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>No/partial resolution</td>
<td>54.9% (n=124)</td>
<td>30.4% (n=28)</td>
<td></td>
</tr>
</tbody>
</table>

g. The effect of age
First, the mean age of male and female participants who experienced full resolution of their symptoms was compared. The mean age of male and female participants was comparable for participants who experienced full resolution loss of smell (40.4 ± 13.2 years in males vs. 38.1 ± 11.3 in females, p=0.333), their loss of taste (40.3 ±13.4 vs .37.1 ± 10.7 p=0.153) and a combined loss of smell and taste (40.0 ± 12.6 vs. 37.1 ± 11.0, p=0.122). The effect of age on resolution of smell and/or taste loss was subsequently evaluated across different age group, as summarised in Table 2.10. Full resolution of their smell and taste symptoms was reported with a higher frequency in participants aged under 50 years, although these differences did not reach statistical significance.

Table 2.10: Smell and or taste loss resolution rates among different age groups in participants with SARS-CoV-2 antibodies

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>Smell loss</th>
<th>Taste loss</th>
</tr>
</thead>
<tbody>
<tr>
<td>18-29</td>
<td>40.2% (n=12)</td>
<td>59.8% (n=41)</td>
</tr>
<tr>
<td>30-49</td>
<td>41.9% (n=71)</td>
<td>58.1% (n=118)</td>
</tr>
<tr>
<td>50-64</td>
<td>47.8% (n=33)</td>
<td>52.2% (n=37)</td>
</tr>
<tr>
<td>65+</td>
<td>30% (n=4)</td>
<td>70% (n=6)</td>
</tr>
</tbody>
</table>

\(^1\) p value adjusted for multiple comparisons

### h. The effect of age and sex

Subsequently, the mean age of male and female with ongoing loss of smell and/or taste were compared. The mean age of female participants with ongoing smell loss was 41.6 ± 11.7 years compared to 37.4 ± 12.7 years in male participants, however this borderline difference did not reach statistical significance (p=0.053). In participants with persistent loss of taste that did not resolve at the time of follow-up, mean age was significantly higher in females compared to males (42.7 ± 12.5
years vs. 37.6 ± 12.6 years, \( p=0.030 \)). Mean age was also significantly higher in female participants with ongoing combined loss of smell and taste loss compared to male participants (42.8 ± 12.5 vs. 34.6 ± 10.4, \( p=0.001 \)) (Figure 2.6).

**Figure 2.6: Age differences in female versus male participants with SARS-CoV-2 antibodies without full smell and/or taste loss resolution.**

Figure comparing age differences in female versus male participants without full smell and/or taste resolution. Mean age was significantly higher in female participants compared to male participants who reported their taste loss had not resolved (42.7 ± 12.5 years vs. 37.6 ± 12.6 years, \( p=0.030 \)) and in female participants with combined smell and taste loss that did not resolve by 4-6 week follow-up (42.8 ± 12.5 vs. 34.6 ± 10.4, \( p=0.001 \)). (*) = statistically significant association. SARS-CoV-2: Severe Acute Respiratory Syndrome Coronavirus 2, SD: Standard deviation.

Given the above findings, the effect of age on smell and/or taste loss resolution in female participants was further examined. A significantly higher age was observed in female participants with ongoing smell loss compared to those with full recovery of their sense of smell (41.6 ± 11.7 vs. 38.1 ± 11.3 years, \( p=0.010 \)). Significant differences in age were also observed in women with persistent compared to resolved taste loss (42.7 ± 12.5 vs. 37.1 ± 10.7 years, \( p<0.001 \)) and combined loss of smell and taste (42.8 ± 12.5 vs. 37.1 ± 11.0 years, \( p<0.001 \)).
2.3.9 Predictors of persistent smell loss in a community population with SARS-CoV-2 antibodies and acute loss of their sense of smell

A logistic regression analysis was performed in order to assess the relative importance of participant’s age, sex, ethnicity, smoking status, presence of parosmia and smell loss pattern as risk factors for persistent smell loss at >4 weeks from onset, as seen in Table 2.11.

Table 2.11: Logistic regression exploring the association between age, sex, ethnicity, smoking status, presence of parosmia and smell loss pattern (complete vs partial) and no resolution of smell loss at 4 weeks follow up.

<table>
<thead>
<tr>
<th>Variable</th>
<th>B</th>
<th>OR</th>
<th>95% CI (lower)</th>
<th>95% CI (upper)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>0.13</td>
<td>1.013</td>
<td>0.994</td>
<td>1.032</td>
<td>0.172</td>
</tr>
<tr>
<td>Ethnicity</td>
<td>0.96</td>
<td>1.101</td>
<td>0.595</td>
<td>2.034</td>
<td>0.760</td>
</tr>
<tr>
<td>Complete smell loss</td>
<td>0.529</td>
<td>1.697</td>
<td>0.998</td>
<td>2.884</td>
<td>0.051</td>
</tr>
<tr>
<td>Parosmia</td>
<td>0.904</td>
<td>2.470</td>
<td>1.539</td>
<td>3.966</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Sex (Female)</td>
<td>0.901</td>
<td>2.461</td>
<td>1.468</td>
<td>4.126</td>
<td>0.001</td>
</tr>
<tr>
<td>Smoking</td>
<td>0.303</td>
<td>1.355</td>
<td>0.604</td>
<td>3.038</td>
<td>0.462</td>
</tr>
</tbody>
</table>

CI: Confidence Interval

Female participants were almost 2.5 times more likely to have ongoing smell loss after 4 weeks compared to participants of male sex (OR 2.46, 95% CI 1.47 to 4.13, p=0.001). Parosmia was also shown to have a significant association with unresolved smell loss at 4-6 week follow-up (OR 2.47, 95% CI 1.54 to 4.00, p<0.001). A complete loss of smell at presentation, had a OR 1.697 (CI 0.998 to 2.884), however this did not reach statistical significance (p=0.051). The model adjusted for age, ethnicity and smoking status.

2.3.10 Predictors of persistent taste loss in a community population with SARS-CoV-2 antibodies and acute loss of their sense of taste

A logistic regression analysis was performed in order to assess the relative importance of participants’ age, sex, ethnicity, smoking status, taste loss pattern, presence of
dysgeusia and unusual taste in the absence of eating, as risk factors for persistent loss of taste at >4 weeks from onset, as seen in Table 2.12.

Table 2.12: Logistic regression exploring the association between age, sex, ethnicity, smoking status, presence of dysgeusia and taste loss pattern (complete vs partial) and no resolution of taste loss at 4-6 weeks follow up.

<table>
<thead>
<tr>
<th>Variable</th>
<th>B</th>
<th>OR</th>
<th>95% CI (lower)</th>
<th>95% CI (upper)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>0.029</td>
<td>1.029</td>
<td>1.009</td>
<td>1.050</td>
<td>0.005</td>
</tr>
<tr>
<td>Ethnicity</td>
<td>0.192</td>
<td>1.212</td>
<td>0.615</td>
<td>2.385</td>
<td>0.578</td>
</tr>
<tr>
<td>Complete taste loss</td>
<td>0.217</td>
<td>1.242</td>
<td>0.764</td>
<td>2.019</td>
<td>0.382</td>
</tr>
<tr>
<td>Dysgeusia</td>
<td>0.213</td>
<td>1.237</td>
<td>0.756</td>
<td>2.025</td>
<td>0.397</td>
</tr>
<tr>
<td>Phantom taste sensation</td>
<td>0.775</td>
<td>2.127</td>
<td>1.235</td>
<td>3.666</td>
<td>0.007</td>
</tr>
<tr>
<td>Sex (Female)</td>
<td>1.076</td>
<td>2.933</td>
<td>1.630</td>
<td>5.278</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Smoking</td>
<td>0.525</td>
<td>1.690</td>
<td>0.724</td>
<td>3.947</td>
<td>0.225</td>
</tr>
</tbody>
</table>

CI: Confidence Interval

Female participants were almost 3 times more likely to have ongoing taste loss after 4-6 weeks from their onset of symptoms (OR 2.933, CI 0.764 to 2.019, p<0.001). Age also had a significant effect (OR 1.029, CI 1.009 to 1.050, p=0.005), as well as the presence of phantom taste sensations (OR 2.127, CI 1.235 to 3.666, p=0.007).

2.3.11 Persistent smell and/or taste loss as a clinical manifestation of long COVID

At the end of the 4-6 week follow-up period 42.3% (n=151) of participants with positive SARS-CoV-2 antibodies had ongoing smell loss, 33.8% experienced ongoing taste loss and 36% had ongoing taste and smell loss, in keeping with a presentation of long COVID.

The resolution of other symptoms of COVID-19 at the end of the follow-up period in participants with SARS-CoV-2 IgG/IgM antibodies was also evaluated. Out of 134 participants with unresolved smell loss who reported additional COVID-19 symptoms
on their original questionnaire, 29.1% (n=39) had at least 1 additional unresolved symptom at the time they completed their follow-up questionnaire, compared to 19.9% (n=35) of participants with full resolution of their smell loss (29.1% vs 19.9%, p=0.059). The most commonly reported unresolved symptoms were shortness of breath, chest pain and muscle/joint pains.

2.3.12 Objective assessment of smell in a subset of participants using the UPSIT

A subsample of 50 participants underwent objective olfactory testing using the UPSIT. In this subgroup, 84% of participants were female (n=42) and 16% (n=8) male. The mean age was 39.6 ±13.5 years and mean duration of test date from the onset of symptoms was 21.6 ±4.7 weeks. 76% (n=38) of participants had complete loss of their sense of smell at the time their original symptoms developed and 24% (n=12) had experienced a partial loss of smell. At the time of the follow-up questionnaire 4-6 weeks later 16% (n=8) reported their smell loss ‘did not resolve’, 42% (n=21) reported their smell loss ‘resolved partially’ and 42% (n=21) reported their smell loss ‘resolved fully’.

UPSIT testing results showed a mean score of 29.1 ±7.5 points. In view of the time elapsed between the completion of the follow-up questionnaire and the date the UPSIT tests were performed, participants were asked how they perceived their smell function prior to undertaking the test. Their answers were grouped into:

- ‘I have no or minimal sense of smell’
- ‘My sense of smell improved but has not fully recovered’
- ‘My sense of smell fully recovered’.

Table 2.13 illustrates a comparison of participants’ perceived smell function and their UPSIT test result, by test result category. A Spearman rank correlation analysis showed a significant correlation between perceived smell function and UPSIT test result category (r=0.84 ± 0.71 to 0.90, p<0.001).
Table 2.13: Comparison of UPSIT test result and perceived smell function

<table>
<thead>
<tr>
<th></th>
<th>Minimal/no sense of smell (n=11)</th>
<th>Sense of smell improved, but not fully recovered (n=9)</th>
<th>Sense of smell fully recovered (n=30)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total anosmia</td>
<td>45.5% (5)</td>
<td>0%</td>
<td>0%</td>
</tr>
<tr>
<td>Severe microsmia</td>
<td>45.5% (5)</td>
<td>0%</td>
<td>0%</td>
</tr>
<tr>
<td>Moderate microsmia</td>
<td>9.0% (1)</td>
<td>77.8% (7)</td>
<td>0%</td>
</tr>
<tr>
<td>Mild microsmia</td>
<td>0%</td>
<td>11.1% (1)</td>
<td>16.7% (n=5)</td>
</tr>
<tr>
<td>Normosmia</td>
<td>0%</td>
<td>11.1% (1)</td>
<td>83.3% (n=25)</td>
</tr>
<tr>
<td>Spearman r</td>
<td>0.95</td>
<td>-0.63</td>
<td>-0.89</td>
</tr>
</tbody>
</table>

2.4 Discussion

2.4.1 Seroprevalence of SARS-CoV-2 antibodies and chemosensory dysfunction as manifestations of COVID-19

Since the outbreak of the COVID-19 pandemic, it has become evident that loss of smell and/or taste are highly prevalent symptoms[15]. The results of this study demonstrated a seroprevalence of SARS-CoV-2 IgG/IgM of 77.6% in a community-based cohort, undertaken during the peak of the first COVID-19 wave in London, UK. At the time the study was undertaken, loss of the smell and taste were not recognised as official symptoms of COVID-19 and people who experienced these symptoms did not meet the local criteria for self-isolation and testing. The finding that 39.8% (n=175) of participants had neither a cough nor a fever, highlighted the importance from a public health perspective, for people with loss of smell and/or taste to be offered testing and be advised to isolate. The study, at the time it was performed, also offered insights into the seroconversion of people with mild symptoms. Given the timing of the study and the fact it included people who were symptomatic early in the course of the pandemic, antibodies to SARS-CoV-2 could not have been due to earlier exposure to the virus, nor to vaccination. In addition, the subjective assessment of smell loss by the participant population in this study, was correlated with an objective assessment of olfactory function using the UK version of the UPSIT test in a subset of 50 participants.
The results demonstrated that UPSIT scores strongly correlated with perceived smell function in this population, which highlights the reliability of our subjective patient-reported data on smell function following COVID-19. This finding is in line with a study conducted in ambulatory COVID-19 patients using the 12-item Brief Smell Identification Test (BSIT) which also concluded that self-reported olfactory loss is a strong predictor of abnormal olfactory function [16].

Importantly, the fact that the study questionnaire included separate questions on smell and taste also enabled a direct comparison of their relationship to SARS-CoV-2 IgG/IgM status. Loss of smell was more prevalent in participants with SARS-CoV-2 antibodies compared to those without antibodies (93.4% vs. 78.7%, p<0.001), whereas taste loss was equally prevalent (90.2% vs. 89.0%, p=0.738). Importantly, participants with loss of smell were 3 times more likely to be seropositive for SARS-CoV-2 IgG/IgM (OR 2.86; 95% CI: 1.27 to 6.36; p<0.001) compared to those with taste loss. These findings indicate that the loss of smell is a more specific and more predictive symptom of COVID-19 compared to the loss of taste, although they were reported with comparable frequency. In addition, combined chemosensory loss of both smell and taste was 4 times more likely to result in positive SARS-CoV-2 antibodies, compared to taste loss alone. It is not surprising that the majority of participants reported combined smell and taste loss, as the processes of chemosensation in taste and smell are highly interlinked. Retro-nasal olfaction, which results in the perception of flavour, is a main element in the signalling processes that result in the perception of the flavour of food. Therefore, loss of an element of taste perception is a natural consequence of smell loss, as flavour perception through retro-nasal olfaction is lost. The comment from this participant (Table 2.3) illustrates a clear description of the loss of retro-nasal olfaction: “I could only taste very basic things like bitterness or sweetness, couldn’t pick up any aromas or flavours in food or drinks.”

However, looking further into the presentations of participants with taste loss in the study, it does become apparent that the cohort of participants with taste loss represents more than one pathological process, in addition to the loss of retro-nasal olfaction. A total of 6.6% of participants with SARS-CoV-2 antibodies reported an
isolated loss in their sense of taste, in the absence of smell loss. In addition, 47.5% of SARS-CoV-2 IgG/IgM positive participants reported a complete loss of their sense of taste. Notably, a complete loss of the sense of taste was significantly more common in participants with positive SARS-CoV-2 antibodies, compared to those without antibodies (p<0.001). Descriptions of complete taste loss from participants suggest a complete loss of chemosensory perception of any food properties beyond textural cues: “I could not taste even the spiciest of foods or sweetest. I tried different chillies too but nothing had a taste.” and “I couldn’t taste chillies or any food. Drinks were just liquid.” (Table 2.3). These findings, taken together, suggest the presence of an alternative pathophysiological mechanism affecting gustatory pathways. The presence of ACE2 on the lingual mucosal epithelium and taste buds themselves provides a plausible explanation[17]. Furthermore, ACE2 is also expressed on the salivary gland and given the role of saliva in taste function, this could explain a degree of gustatory dysfunction in COVID-19[18]. Finally, direct damage to the cranial nerves involved in gustatory signalling provides an alternative explanation[19].

Interestingly, several of the study participants were specific in their comments that their symptoms occurred in the absence of nasal congestion (i.e., “It was unlike when I have had similar experiences with colds because my nasal passages were not blocked and I could breathe normally.”). Although multiple upper respiratory tract viruses can result in impaired olfactory function, the characteristic feature of smell loss due to SARS-CoV-2, is that is appears in the absence of any nasal congestion, rhinitis or rhinorrhoea[20-22]. In line with this observation, Huart et al., undertook a comparative study between patients with olfactory impairment due to COVID-19 versus other upper respiratory tract viruses using psychophysical olfactory and gustatory testing. Their results illustrated a different pattern of chemosensory loss in COVID-19 compared to other upper respiratory tract infections, with reduced discrimination and identification performances in COVID-19 patients, which suggests involvement of central mechanisms in COVID-19[23].

Following the emerging reports of COVID-19 related anosmia, a number of hypotheses emerged in an attempt to the underlying pathophysiology[24]. A number of studies
reported findings of olfactory epithelial injury in COVID-19. Combined with the fact that olfactory sensory neurones don’t express ACE2 receptors, whereas this is expressed on epithelial cells in the olfactory mucosa, led to the hypothesis that anosmia is a result of injury to the olfactory epithelium, which in turn leads to olfactory cleft inflammation resulting in a conduction loss of the olfactory stimulus. Patients with COVID-19 related anosmia have been shown to have higher olfactory cleft widths and volumes compared to control subjects[25]. Indeed, in a study of 20 SARS-CoV-2 patients with anosmia had 3T MRI, performed both at the early stage of the disease and at the 1-month follow-up. MRI displayed a complete obstruction of the olfactory cleft in 19 of 20 patients. At 1-month follow up, 7 of 20 patients still had an obstruction of the olfactory cleft, which correlated with olfactory function[26]. Lechien et al., however in a study assessing the olfactory cleft of patients with COVID-19 anosmia, demonstrated no olfactory cleft abnormality in the majority of patients, suggesting that this might cause anosmia in a subset of patients with short-lived anosmia, but does not explain olfactory impairment in the majority of cases[24]. In line with the hypothesis that olfactory epithelial cell infection via ACE2 cell entry is the primary process resulting in smell loss, a further theory suggested that injury to these cells gives rise to a sensorineural impairmen[27]. Evidence from animal, post-mortem studies and nasal epithelial biopsies suggest inflammatory cell invasion and atrophy of the olfactory epithelium[28-30]. These studies suggest damage to olfactory epithelial support cells as a pathophysiological mechanism. In addition, these findings also suggest that, through indirect consequences, SARS-CoV-2 results in loss of function of the olfactory bulb.

Although the effects of SARS-CoV-2 on the olfactory epithelium have been illustrated, the neurotropic properties of the virus are now also evident. However, cell entry via the ACE2 receptor does not fully explain the neurotropic properties of SARS-CoV-2 and a number of alternative mechanisms have recently been proposed. In addition to ACE2, the SARS-CoV-2 spike protein has also been demonstrated to bind to the neuropilin-1 receptor (NRP1), facilitating cell entry and infectivity[31,32]. NRP1 is expressed on all olfactory cells, including olfactory sensory neurones and their progenitor cells, as well as regulatory T cells[31,32]. This poses a plausible route for SARS-CoV-2 cell entry into olfactory neurones and potentially higher CNS centres. Although the above proposed
hypotheses are by no means mutually exclusive, a more recent body of MRI data in the COVID-19 population further supports the ability of SARS-CoV-2 to directly invade the CNS. Our findings of a high percentage of participants with SARS-CoV-2 antibodies reporting complete loss of smell compared to participants without antibodies (69.8% vs 39.4%, p<0.001), taken together with the descriptions illustrated in Table 2.3, support the hypothesis of SARS-CoV-2 affecting the olfactory tract at the level of the CNS. Furthermore, our participant population also commonly reported a number of other symptoms suggestive of CNS involvement, including parosmia, dysgeusia, the presence of taste sensations without eating, headaches, confusion, drowsiness and disorientation.

2.4.2 The clinical course of chemosensory dysfunction in COVID-19 and risk factors for persistent symptoms

This study is one of the first to investigate the clinical course and resolution of acute smell and/or taste in a community cohort with and without SARS-CoV-2 IgG/IgM antibodies. The study’s findings demonstrate a higher recovery rate of smell loss (72.1% vs. 57.7%; p=0.027), taste loss (80.3% vs. 66.2%; p=0.017) and combined smell and taste loss (79.6% vs. 64%; p=0.026) in participants negative for SARS-CoV-2 IgG/IgM antibodies compared to those positive for SARS-CoV-2 IgG/IgM antibodies. Interestingly, a study by Beltrán-Corbellini et al., compared recovery after smell loss in 70 COVID-19 and 40 influenza patients[33]. Forty percent of COVID-19 positive participants reported full recovery after 7.4 ±2.3 days and 16.7% partial recovery after 9.1 ±3.6 days, whereas 100% of influenza patients had fully recovered their sense of smell within the same follow-up window. In the same study, COVID-19 patients also had higher rates of complete smell loss, in line with the hypothesis that COVID-19 is a different and more severe pathophysiologial entity, compared to post-viral smell loss from other respiratory pathogens[33].

Importantly, this study illustrates a high percentage of participants with persistent smell loss (42.3%), taste loss (33.8%) and combined smell and taste loss (36.0%) after a 4-6 week follow-up period. Similarly, a longitudinal study of 751 patients with COVID-19 diagnosed in a hospital setting on the basis of either PCR or IgG/IgM antibodies,
reported that after a mean follow up time of 47 days, 37% of patients still had ongoing smell loss[34]. The rate of smell loss resolution rate of 57.7% in participants with SARS-CoV-2 antibodies within 4-6 weeks in this study is consistent with the existing literature[6,35]. Dell’Era et al., reported that 62.9% of participants with smell and/or taste loss during at the time of their COVID-19 infection reported full resolution of both sense of smell/taste symptoms at a follow up assessment carried out at a median time of 23 days (range 15-31)[36]. In contrast to our community-based study, however, their findings come from a hospitalised patient cohort. Resolution rates within approximately 3 months from the onset of symptoms in the literature currently range from 29-92.8%, with discrepancies likely due to differences in study populations, sample size, location and duration of follow-up[5,6,37].

Resolution of smell and/or taste symptoms in this study were higher in people with a partial loss of smell and/or taste, compared to those who experienced complete sensory modality loss. Prolonged recovery times in patients with complete compared to partial loss of smell were also reported by Kosugi et al.[38]. A study undertaking objective evaluation of olfactory function also found that a higher baseline severity of smell loss was strongly predictive of persistent smell loss[39]. The prolonged recovery times for participants with complete loss of smell and/or taste further supports a more central pathophysiological mechanism.

Among the key findings of this study is the relationship between distorted chemosensory function and persistent smell and taste dysfunction in participants with positive SARS-CoV-2 antibodies at the end of the study’s follow-up period. Fewer participants with parosmia at the time of their original symptoms had recovered their sense of smell compared to participants who did not experience this (41.4% vs. 65%, p<0.001). Similarly, with regard to recovery of taste loss, lower recovery rates were seen in participants with dysgeusia (60.7% vs. 71.4%, p=0.036) and in participants who experienced phantom taste sensations compared to participants who did not (51.2% vs 71.0%, p=0.001). Furthermore, these symptoms also had predictive value with regard to persistent smell or taste loss at the end of the follow-up period. Parosmia was identified as a strong predictor of persistent smell loss at the end of 4-6 weeks (OR
2.470, CI 1.539 to 3.966, p<0.001) and experiencing unusual tastes in the absence of eating or drinking a predictor for persistent taste dysfunction (OR 2.127, CI 1.235 to 3.666, p=0.007). Parosmia had already been linked to ongoing post-viral smell loss prior to the COVID-19 pandemic[40]. Parosmia has been associated with both a decreased number and disordered regrowth of olfactory axons into existing neural circuits and a preponderance of immature neurons[41]. In contrast, Liu et al. in a study including 153 patients, demonstrated that parosmia was associated with clinically significant recovery in patients receiving olfactory training[42]. Therefore, historically in the context of post-viral smell loss, parosmia has been linked to smell impairment, but has also been viewed as a sign of recovery. Its role as a prognostic marker remains largely unclear. However, findings from this study suggest that parosmia is a marker of poor prognosis in COVID-19. Chemosensory distortions, including phantom taste experiences have also been reported to be more prevalent in patients with positive COVID-19 tests results[43]. Although their underlying mechanism and prognostic value are not fully understood, neurophysiology studies suggest that phantom taste experiences are a result of cranial nerve and particularly chorda tympani dysfunction[44,45]. Together, these findings suggest that distorted chemosensory perception is a risk factor for prolonged smell and/or taste loss and long-COVID.

Furthermore, significant age and sex differences were evident with regard to recovery of smell and/or taste function. Female participants reported lower resolution rates for smell loss (51.4% vs. 72.8%; p<0.001), taste loss (60.1% vs. 80.8%; p<0.001) and combined smell and taste loss (45.1% vs. 69.6%; p<0.001) compared to male participants. Furthermore, female sex was a predictor for persistent smell loss and persistent taste loss in the logistic regression analyses. Prolonged recovery times following smell loss in females with COVID-19 are also reported in the literature[21,36]. Although in the study by Meini et al., the recovery rate did not differ significantly between males and females, the mean recovery time from smell/taste loss was significantly longer for females than for males (26 vs. 14 days, p=0.009), even though the mean age of male participants in the study was significantly higher than that of females (66 vs. 57 years, p=0.04)[35]. Parente-Arias et al., also reported poorer recovery rates in female patients[46].
In this study, female participants with persistent smell loss were significantly older (41.6 ± 11.7 years) than female participants who reported full resolution of smell loss (38.1 ± 11.3 years, p=0.010). The same was also found to be true for taste loss (42.7 ± 12.5 vs. 37.1 ± 10.7, (p<0.001) and combined smell and taste loss (42.8 ± 12.5 vs. 37.1 ± 11.0, p<0.001). Increasing age has also been associated with lower recovery rates of smell and taste dysfunction in other COVID-19 follow-up studies, however these findings are not consistent[35,46]. Lee et al. reported that young age, particularly the age group of 20-39 years, showed a tendency to be associated with a longer persistence of anosmia[47]. Methodological differences, as well as recruitment setting, such as hospital versus community cohorts, are likely to underlie some of these discrepancies between those results. With regard to potential age-related physiological drivers, oestradiol has been shown to increase olfactory epithelial cell density and to have protective role effects against declines in olfactory function[48]. Furthermore, in animal models of neurogenerative diseases oestradiol replacement prevents olfactory dysfunction[49]. This study was not powered to estimate the effect of menopause-driven differences in this subgroup. Nevertheless, a link between persistent chemosensory dysfunction and a post-menopausal state appears plausible.

### 2.4.3 Chemosensory dysfunction as a manifestation of long COVID

It is now evident that loss of smell and/or taste can persist beyond the resolution of the acute phase of a COVID-19 infection[50]. The relatively short follow-up window for this study, combined with the early stages of post-COVID follow-up experience in clinical settings, illustrate that it still remains too early to conclude the exact time frame for prolonged recovery of chemosensory function or whether this may result in permanent smell and/or taste loss in a subset of patients. Data from the clinical course of smell loss from respiratory tract viruses, could persist for two or more years[51,52]. Irrespective however of the long-term prognosis, loss of smell and taste undoubtedly constitute a manifestation of long-COVID, which may result in significant psychological morbidity and adversely impact quality of life[53-55].
In addition, given the limited accessibility to testing in the earlier phases of the pandemic, further patient cohorts may present with olfactory and gustatory impairment and features of long-COVID, as clinical services continue to increase their capacity, indicative of previous COVID-19 infections. Of note, current proposed diagnostic criteria for long-COVID or chronic COVID-19 do not necessitate a positive test at time of symptom onset. The magnitude of the pandemic and the potential for SARS-CoV-2 to cause long-term smell and/or taste dysfunction in relatively small cohorts therefore suggest that the overall prevalence of long-COVID with chemosensory dysfunction within the general population will be considerable[56,57]. It will therefore be essential to improve our understanding of the pathophysiological mechanisms, in order to improve the range and efficacy of available treatments. Therapeutic strategies including olfactory rehabilitation and corticosteroids are already in use to aid recovery of smell function following COVID-19, with further therapeutic strategies in clinical trials [3,58]. Furthermore, in light of the potentially large patient populations that may present with chemosensory dysfunction following COVID-19, accurate prognostication tools and risk stratification will be required, in order to be able to prioritise higher-risk patients earlier in the disease process.

2.4.4 Limitations

This study has a number of limitations, some of which were addressed during the course of the study. The main limitation of this study is the lack of a general population control group without loss of smell and/or taste. The study only recruited participants who reported acute smell and/or taste loss. Whereas this allowed us to study this presentation and its relevance to COVID-19, this also presents a degree of selection bias; hence our findings refer to a population subset with new acute loss of smell and/or taste. Additionally, the web-based delivery of the study, results in susceptibility to a degree of selection and age bias, as participation required proficiency with computers and smartphones and may have resulted in under-representation of older adults. Furthermore, the majority of our participants were female; this may reflect previous findings that females are more likely to engage in research and also have a higher frequency of loss of smell and/or taste with COVID-19 than males.
The study used self-reported data on symptoms of smell and/or taste loss, however this limitation was addressed in part as the telemedicine interviews allowed a clinician member of the study team to validate participants’ responses during the conversation. Furthermore, this was further addressed by recruiting a subset of participants for objective testing, which demonstrated a strong correlation between perceived and assessed smell function.

2.5 Conclusions
The FORECAST study demonstrated that in a community-based cohort of people with acute smell and/or taste loss, 77.6% were seropositive for SARS-CoV-2 IgG/IgM antibodies. Loss of smell was a more specific symptom that loss of taste, with smell loss being 3 times more likely to result in IgG/IgM seropositivity. The study also followed up this community cohort over a 4-6 week period and illustrated that although smell and/or taste loss is a transient phenomenon in the majority SARS-CoV-2 cases, chemosensory dysfunction continues to affect over a third of this patient population 4-6 weeks after the acute phase of COVID-19. The study identified parosmia and female sex as risk factors for persistent smell loss, as well as increasing age within the female sub-cohort. Similarly, female sex and increasing age and distorted taste perception were associated with persistent taste dysfunction.

Persistent smell and taste loss constitute features of long-COVID. The population of patients with longstanding smell and/or taste loss as part of long-COVID will continue to grow during and following the pandemic. Given the impact of these symptoms on quality of life and safety, support and treatment pathways will be essential. The findings of this study suggest that female patients, with distorted chemosensory function are likely to benefit from therapeutic interventions to prevent persistent smell and/or taste loss and should be prioritised when targeted therapies for post-covid smell and taste loss become available. However, offering more targeted treatments will rely on further elucidating the pathophysiological mechanisms that underly these symptoms, which ongoing and future studies will aim to achieve. The work in Chapter 3, will offer some insights into the neurological pathogenesis of olfactory dysfunction at the level of the central nervous system.
2.6 Chapter 2 References

1. ENT UK. Loss of sense of smell as marker of COVID-19 infection [Available from: https://www.entuk.org/sites/default/files/files/Loss%20of%20sense%20of%20smell%20as%20marker%20of%20COVID.pdf].


### 2.7 Chapter 2 Appendix

#### 2.7.1: Participant demographics questions

<table>
<thead>
<tr>
<th>Question</th>
<th>Answer field</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Date of birth</td>
<td>DD/MM/YYYY</td>
</tr>
<tr>
<td>2. Sex</td>
<td>Female, Male, Other</td>
</tr>
<tr>
<td>3. Ethnicity</td>
<td>White, Black, Asian, Mixed, Other, I do not wish to disclose</td>
</tr>
<tr>
<td>4. Have you ever smoked?</td>
<td>Current smoker, Ex-smoker, Never smoked</td>
</tr>
</tbody>
</table>

#### 2.7.2: Participant symptom questionnaire

<table>
<thead>
<tr>
<th>Question</th>
<th>Answer field</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Have you noticed any changes in your sense of smell?</td>
<td>Yes, No</td>
</tr>
<tr>
<td>1.2 How would you describe the change in your sense of smell?</td>
<td>- I can/could no longer smell any odours that I used to be able to smell</td>
</tr>
<tr>
<td></td>
<td>- I can/could no longer smell some odours I used to be able to smell</td>
</tr>
<tr>
<td></td>
<td>Describe (optional)</td>
</tr>
<tr>
<td>1.3 Have you noticed that odours smell different than they used to?</td>
<td>No, Yes, If yes describe (optional)</td>
</tr>
<tr>
<td></td>
<td>Question</td>
</tr>
<tr>
<td>---</td>
<td>--------------------------------------------------------------------------</td>
</tr>
<tr>
<td>2</td>
<td>Have you noticed any changes in your sense of taste?</td>
</tr>
<tr>
<td>2.1</td>
<td>How would you describe the changes in your sense of taste?</td>
</tr>
<tr>
<td>2.2</td>
<td>Have you noticed that foods and drinks taste differently?</td>
</tr>
<tr>
<td></td>
<td>If yes describe (optional)</td>
</tr>
<tr>
<td>2.3</td>
<td>Have you experienced any unusual tastes while not eating or drinking?</td>
</tr>
<tr>
<td></td>
<td>If yes describe</td>
</tr>
<tr>
<td>3</td>
<td>Have you experienced any of these symptoms in the last 4 weeks?</td>
</tr>
<tr>
<td>4</td>
<td>Has/was your appetite for food decreased?</td>
</tr>
<tr>
<td>5</td>
<td>Have you had a test for COVID-19?</td>
</tr>
</tbody>
</table>
### Part 2.7.3: Participant follow-up questionnaire

<table>
<thead>
<tr>
<th>Question</th>
<th>Answer field</th>
<th>Participant number</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>1</strong> Did the changes in your sense of smell resolve?</td>
<td>Resolved fully</td>
<td>Date: Resolved partially</td>
</tr>
<tr>
<td><strong>2</strong> Did the changes in your sense of taste resolve?</td>
<td>Resolved fully</td>
<td>Date: Resolved partially</td>
</tr>
<tr>
<td><strong>3</strong> Did your other symptoms resolve?</td>
<td></td>
<td></td>
</tr>
<tr>
<td>If yes please enter the date these resolved</td>
<td>Cough</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Fever (37.8°C or more)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Shortness of breath</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Chest pain/tightness</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Headache</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Sore throat</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Hoarse voice</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Abdominal pain</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Diarrhoea</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Vomiting</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Confusion, disorientation or drowsiness</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Muscle/joint aches</td>
<td></td>
</tr>
<tr>
<td><strong>4</strong> Have you developed any of the following symptoms in the last 4 weeks?</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Cough</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Fever (37.8°C or more)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Shortness of breath</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Chest pain/tightness</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Headache</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Sore throat</td>
<td></td>
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<tr>
<td></td>
<td>Hoarse voice</td>
<td></td>
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<tr>
<td></td>
<td>Abdominal pain</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Diarrhoea</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Vomiting</td>
</tr>
<tr>
<td>---</td>
<td>---------------------</td>
<td>----------</td>
</tr>
<tr>
<td>5</td>
<td>Have you attended/been admitted to hospital due to COVID-19?</td>
<td>Yes</td>
</tr>
</tbody>
</table>
3. Chapter 3: MRI imaging in the assessment of COVID-19 related anosmia

3.1 Introduction

Anosmia is now an established symptom of COVID-19. Although a growing body of evidence supports the fact that the SARS-CoV-2 virus is neurotropic, the exact pathophysiology through which SARS-CoV-2 impairs olfactory function is not known[1]. Post-mortem studies have revealed the presence of SARS-CoV-2 RNA and viral proteins within brain tissue of deceased COVID-19 patients, including the olfactory bulb[2,3]. Neurological symptoms and syndromes, including encephalitis, demyelinating disease, cerebrovascular disease and neuropathies have been described in the COVID-19 patient population[4]. Furthermore, neurological manifestations of long-COVID, including anosmia, fatigue, difficulties concentrating and cognitive decline are commonly reported long COVID manifestations, even in people with mild clinical disease during the acute infectious phase[5].

A number of studies and clinical case series have used clinical MRI in COVID-19 patients with ongoing anosmia and demonstrated evidence of olfactory bulb abnormalities, including olfactory oedema and atrophy[6-8]. Olfactory bulb oedema, measured from T2 weighted anatomical images, has been seen in patients who had previously normal brain appearances from MRI scans and has been shown to resolve following resolution of anosmia[8,9]. In contrast, olfactory bulb atrophy has been demonstrated in patients with persistent olfactory dysfunction after 40 days from the onset of symptoms, suggesting that atrophy could be a more long-standing pathological consequence in patients with persistent anosmia[10]. Furthermore, a case report has illustrated olfactory hyperintensities and linear hyperintensities inside bilateral olfactory nerves, suggesting olfactory neuropathy in a patient with COVID-19 related anosmia[11]. Taken together, these findings suggest that SARS-CoV-2 CNS invasion occurs, at least in part, through the olfactory tract. Although the ability of SARS-CoV-2 to invade nervous tissue and CNS structures is evident, the extent of direct and indirect effects
of SARS-CoV-2 invasion within the CNS and the implications for long-term pathology and recovery are not known.

To date, most of the COVID-19 neuroimaging literature stems from clinical case series of predominantly acute cases, using qualitative MRI. However, microstructural quantitative MRI (qMRI), which is aimed at detecting changes occurring at a scale smaller than the image resolution, can provide information on alterations within brain tissues beyond clinically identifiable lesions[12]. Few studies have used quantitative brain imaging to date to assess the neuropathogenesis of SARS-CoV-2 in patients who experience smell and/or taste loss. A study on 23 participants with persistent COVID-19 related smell loss, demonstrated that over 90% of participants had olfactory bulb abnormalities, manifested as increased signal intensity, scattered hyperintense foci or microhaemorrhages[13]. In the same study, olfactory cortical abnormalities were also present in a subset of patients[13]. In a qMRI study including 60 patients who recovered from COVID-19, 55% of which experienced neurological symptoms, and 39 controls detected changes in grey matter, which comprises unmyelinated neuronal cell bodies and glial cells, in people who recovered from COVID-19, with reduced grey matter volumes in the right cingulate gyrus and left hippocampus in participants who had anosmia during COVID-19[14]. Douaud et al., in a large-scale biobank follow-up study, invited participants with previous MRI results recorded in the biobank, who had COVID-19 for qMRI imaging in order to assess the longitudinal effects of COVID-19 on the CNS[15]. Their results from 394 participants, demonstrated a loss of grey matter in limbic cortical areas directly linked to the primary olfactory and gustatory system[15]. Although these finding provide compelling evidence for the neuroinvasivity of SARS-CoV-2 via the olfactory and gustatory system, no data are available on the symptomatology of the participants in this study, neither of symptom resolution at the time of imaging. We set out to perform qMRI on a participant cohort from the FORECAST study (Chapter 2) with persistent anosmia and an age- and sex-matched group of participants who fully recovered their olfactory function.

The exploratory work presented in this chapter aimed to use a detailed quantitative MRI protocol to perform brain imaging in people with:
• **Group 1:** SARS-CoV-2 antibodies and persistent anosmia

• **Group 2:** SARS-CoV-2 antibodies with recovered anosmia (age and sex matched to group 1)

• **Group 3:** A cohort of healthy volunteers who remained asymptomatic since the beginning of the COVID-19 pandemic with a negative SARS-CoV-2 antibody test (age and sex-matched to groups 1 and 2)

### 3.2 Methods

#### 3.2.1 Participants and recruitment

For recruitment into study groups 1 and 2, participants who completed the FORECAST Study, described in Chapter 2, who tested positive for SARS-CoV-2 IgG/IgM and provided consent to be contacted for future research studies were contacted after the original study concluded. Participants who expressed an interest in taking part were sent an information sheet, and were subsequently screened for suitability based on the study’s inclusion and exclusion criteria by a trained healthcare professional. Written informed consent was obtained using an electronic consent form.

The study inclusion criteria were:

- Participants who completed the general public FORECAST and consented to be contacted for further research.
- Positive SARS-CoV-2 IgG/IgM antibody test as part of the FORECAST study.
- Being right-handed (for purposes of MRI data analysis).
- Absence of any contraindications to MRI scanning.
- Body weight less than 145kg due to limitations of the MRI scanner.
- Able to lie flat for 1 hour (duration of MRI imaging protocol).

The study’s exclusion criteria were:

- Contraindications specific to MRI scanning: aneurysm clips, pacemakers or any other forms of metallic implants that are not MRI safe.
• Previous diagnosis of a neurological condition that is likely to impact upon imaging analysis, such as CNS structural lesions and demyelinating disease.
• Severe claustrophobia
• Pregnancy or lactation.

Participants who consented to take part were originally sent a UPSIT test (MediSense, www.smelltest.eu), a 40-item ‘scratch and sniff’ test, which has been validated for self-administration, as described in Chapter 2 (UPSIT Cohort)[16]. The UPSIT generates a score out of 40, which following adjustment for age and sex, classifies olfactory function into normosmia, mild microsmia, moderate microsmia, severe microsmia and total anosmia[16]. Participants with total anosmia and severe microsmia were classed as having persistent anosmia and were invited to attend for an MRI scan. Participants with persistent anosmia formed study group 1 and were recruited into the study first. An age- and sex-matched group of participants with smell loss that fully recovered, as assessed by an age and sex-adjusted UPSIT score indicating normosmia, were subsequently also invited for an MRI scan.

A cohort of healthy volunteers who remained asymptomatic since the beginning of the COVID-19 pandemic were recruited into an age and sex-matched control group (Group 3). Participants were sent an information sheet detailing the study and written informed consent was obtained. Recruited participants were tested for SARS-CoV-2 antibodies, using a serology assay for SARS-CoV-2 IgG/IgM antibodies. The inclusion criteria were a negative SARS-CoV-2 antibody test and exclusion criteria for MRI as outlined above.

3.2.2 Ethical approval
The FORECAST study received ethical approval by the Queens’ Square Research Ethics Committee (IRAS Project ID 282668, ClinicalTrials.gov: NCT04377815) and was conducted in line with the declaration of Helsinki and the standards of Good Clinical Practice. The ‘Development of Novel MRI Techniques for Neurological Applications’ study, which recruited participants in Group 3, received ethics approval from the
London Harrow Research Ethics Committee (05/Q0502/101) and was conducted in line with the declaration of Helsinki and the standards of Good Clinical Practice.

### 3.2.3 MRI protocol

Imaging was undertaken at the University College London (UCL) Centre for Neurology, using a Philips Ingenia CX 3T MRI scanner with a 32-channel head coil. The entire MRI protocol used for this study was extensive and consisted of multiple sequences that interrogated both structural and functional characteristics. The total scan time was approximately 1 hour (53min 21sec). Table 3.1 illustrates the sequences, modalities and image-acquisition times for the parameters presented in this chapter. The exact acquisition parameters used have been previously described by Battiston et al.[17].

For this chapter, the focus is on the structural data acquired from the following sequences:

- Clinical 3D T2-weighted fluid-attenuated inversion recovery (FLAIR) sequence implemented to detect clinical abnormalities. T2 images serve as a map of proton energy within fat- and water-containing tissues, therefore cerebrospinal fluid (CSF) and other water-containing structures appear white on T2 images[18].
- High resolution (1x1x1mm³) 3D T1-weighted acquisitions for brain volume measurements from different tissue types and regions. T1 images serve as a map of proton energy within fat-containing tissues of the body, these therefore appear white, in contrast to water which appears dark[18].
- A multi-shot Inversion recovery sequence was acquired with multiple inversion times
- Multi-shell diffusion-weighted imaging (DWI) for microstructure assessment.
- Quantitative magnetization transfer (qMT) for bound pool fraction and macromolecular T (T2b).
Table 3.1: MRI Acquisition protocol

<table>
<thead>
<tr>
<th>Imaging sequences</th>
<th>Spatial Resolution (mm³)</th>
<th>Acquisition time (mm:ss)</th>
<th>Biophysically Meaningful Features</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inversion recovery</td>
<td>2x2x2</td>
<td>4:20</td>
<td>T1 Values</td>
</tr>
<tr>
<td>Magnetisation transfer</td>
<td>2x2x2</td>
<td>4:57</td>
<td>qMT</td>
</tr>
<tr>
<td>B1 mapping</td>
<td>2x2x2</td>
<td>1:00</td>
<td>B1 map used for qMT computation</td>
</tr>
<tr>
<td>Diffusion Weighted Imaging</td>
<td>2x2x2</td>
<td>8:36</td>
<td>Diffusion metrics – FA, MD, ODI</td>
</tr>
<tr>
<td>Clinical Imaging (3D T1)</td>
<td>1x1x1</td>
<td>1:00</td>
<td>Brain tissue volumes</td>
</tr>
<tr>
<td>Clinical Imaging (3D T2 FLAIR)</td>
<td>1x1x1</td>
<td>3:20</td>
<td>Hyperintensities and lesion load</td>
</tr>
<tr>
<td>3D Fast Field Echo</td>
<td>1x1x1</td>
<td>4:06</td>
<td>Macromolecular tissue volume, T2, susceptibility</td>
</tr>
</tbody>
</table>

qMT: quantitative Magnetization Transfer, FA: Fractional Anisotropy, MD: Mean Diffusivity, ODI: Orientation Dispersion Index.

3.2.4 Image processing

Data was automatically transferred from the scanner to a local XNAT server. Initial processing and quality control was conducted in order to identify any lesions and calculate lesion volume prior to analysis. To this end, 3D T2 FLAIR images were assessed for hyperintensities and clinically significant abnormalities by an experienced neuroradiologist. In addition, 3D T1-weighted images were first processed with the NicMSLesion software to detect abnormalities[19]. Images were subsequently reviewed by a trained clinician and manually segmented. The resulting lesion masks were used for extracting Normal Appearing White Matter (NAWM) voxels as well as to perform lesion-filling of the 3D T1-weighted scans before segmentation and registration[20].
3.2.4.1 Volumetric imaging processing

T1 weighted images, which provide qualitative data, were used to calculate volumetric measurements. To permit analysis of volumes across a group, where brain anatomy varies across each person, the individual anatomical images are first warped and scaled to a template space, the Montreal Neurological Institute (MNI) template, a process which is known as registration or normalisation. Once registered to this template space anatomical regions were segmented and the volumes of each of these regions are calculated using an automated segmentation tool[21]. The regions chosen for the subsequent whole brain analysis following segmentation were: grey matter (GM), cortical grey matter (CGM), deep grey matter (DGM), white matter (WM), CSF, brain parenchymal fraction and lesion volume. Furthermore, volumetric results were also compared in the following regions of interest (ROI): Amygdala, brainstem, hippocampus, lateral ventricles, inferior temporal gyrus, right parahippocampal gyrus, anterior insula, and posterior insula. Volumes calculated in mm³ for each tissue type were used for statistical analysis as described below.

3.2.4.1 Quantitative imaging processing

For quantitative T1 measurements, the T1 value of each voxel was calculated from the output of the inversion recovery echo planar imaging (EPI) sequence. Prior to calculation the image series was denoised using the Marchenko-Pastur Principal Component Analysis (MP-PCA) method[20]. Full methods for the T1 mapping calculation are described by Battiston et al.[22]. Similar to the T1-weighted images described above, the calculated T1 maps for each individual were registered to MNI space and the anatomical segmentations, calculated from the steps above, were used as masks to extract mean T1 values (measured in seconds) from each of the tissue types.

qMT values, including bound pool fraction and T2b were calculated as described by Battiston et al.[23]. Whole brain maps were registered to MNI space using the same methods as described above for the T1-weighted anatomical images.
The following diffusion metrics were calculated using in-house software and calculations were calculated as described by Cicarelli et al. [24]: Fractional anisotropy (FA), Mean diffusivity (MD), Orientation Dispersion Index (ODI) and Isotropic diffusion volume fraction (VFISO).

For all the quantitative metrics values extracted from GM, CGM, WM, brainstem and lesions were used in the analysis. Fully automated pipelines, developed by the UCL institute of Neurology, were used for each modality to provide biophysically meaningful features, calculated at voxel and regional levels (Figure 3.1).

**Figure 3.1: Examples of the biophysically meaningful features calculated from the MRI protocol**

![Biophysically meaningful features](image)

Figure displaying images from biophysically meaningful features obtained from the MRI protocol used. MD: Mean Diffusivity, FA: Fractional anisotropy, MK: Mean Kurtosis, ODI: Orientation Dispersion Index, QSM: Quantitative Susceptibility Mapping, BPF: Bound Pool Fraction, ICF: Intensity Curvature Fraction, MTV: Macromolecular tissue volume, CBF: Cerebral Blood Flow.

**3.2.5 Statistical analysis**

Statistical analysis was conducted using SPSS Version 25 (IBM Statistics). Results are expressed as calculated means with SD. The Gaussian distribution was assessed by D’Agostino-Pearson normality test. Comparisons between groups 1 and 2 were made using parametric (t-tests) or non-parametric tests were used as appropriate. Analysis
of Variance (ANOVA) and chi square tests were used to compare demographics between the study groups and for ROI analysis between groups. In view of the fact that MRI results presented in this Chapter remain exploratory, no adjustment for multiple comparisons was carried out between imaging modalities. In order to enable comparisons between all three study groups, linear regression analyses were performed. A general linear model was used, adjusting for age and sex as co-variants with post-hoc analyses.

3.3 Results

3.3.1 Participant demographics
Following review of the FORECAST database and UPSIT testing, 10 participants were identified from the FORECAST study with persistent anosmia, 9 of which agreed to attend for MRI scanning. A total of 10 participants were included into Groups 2 and 3 respectively. The demographics of participants in the three study groups are seen in Table 3.2.

<table>
<thead>
<tr>
<th></th>
<th>Age</th>
<th>Sex</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1: Persistent anosmia group (n=9)</td>
<td>52.3 ±12.2</td>
<td>88.9% female</td>
</tr>
<tr>
<td>Group 2: Resolved anosmia group (n=10)</td>
<td>49.7 ±8.3</td>
<td>90% female</td>
</tr>
<tr>
<td>Group 3: SARS-CoV-2 negative group (n=10)</td>
<td>43.5 ±13.8</td>
<td>70% female</td>
</tr>
<tr>
<td>p value</td>
<td>0.263</td>
<td>0.453</td>
</tr>
</tbody>
</table>

SARS-CoV-2: Severe Acute Respiratory Syndrome Coronavirus 2.

Upon completion, all clinical MRI scans obtained via the study were reviewed by a clinical neuroradiologist. Two scans, one from a participant in Group 1 and a further from a participant in Group 2 were reported to have clinically significant abnormalities. The scan from the participant in Group 1 was reported as having widespread bilateral white matter hyperintensities. The scan from the participant in Group 2 detected bilateral demyelinating lesions. Both these participants were referred to a neurologist.
and it remains unclear if the abnormalities detected were a manifestation of COVID-19. These two participants were excluded from qMRI analysis.

Results from the volumetric and qMRI analyses respectively, are presented first as a comparison between participants in Group 1 versus Group 2, i.e., participants with persistent versus recovered anosmia from the FORECAST study. Subsequently results from the analysis comparing metrics from all three groups of participants are presented.

3.3.2 Volumetric analysis

3.3.2.1 Persistent versus recovered anosmia whole brain analysis

Volumetric MRI measurements from different CNS parameters were compared between participants with persistent versus recovered anosmia (Table 3.3). No differences were seen in GM, CGM, DGM, WM or CSF volumes. However, a significant volume measurement difference was detected between participants with persistent versus recovered anosmia in brain parenchymal fraction (0.745$\text{mm}^3 \pm 0.007$ vs 0.754$\text{mm}^3 \pm 0.011$, $p=0.027$).
Table 3.3: Volumetric analysis between participants with persistent and recovered anosmia

<table>
<thead>
<tr>
<th></th>
<th>Persistent anosmia (n=8)</th>
<th>Recovered anosmia (n=10)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>GM Volume</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean (mm$^3$)</td>
<td>622087 ± 49814</td>
<td>628242 ± 40959</td>
<td>0.778</td>
</tr>
<tr>
<td>SD</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CGM volume</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean (mm$^3$)</td>
<td>586839 ± 47453</td>
<td>593099 ± 38118</td>
<td>0.760</td>
</tr>
<tr>
<td>SD</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DGM volume</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean (mm$^3$)</td>
<td>35248 ± 2934</td>
<td>35143 ± 3180</td>
<td>0.944</td>
</tr>
<tr>
<td>SD</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>WM volume</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean (mm$^3$)</td>
<td>447710 ± 27132</td>
<td>449027 ± 48692</td>
<td>0.946</td>
</tr>
<tr>
<td>SD</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CSF volume</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean (mm$^3$)</td>
<td>366834 ± 33175</td>
<td>350312 ± 16440</td>
<td>0.185</td>
</tr>
<tr>
<td>SD</td>
<td>0.01107 ± 0.01322</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Brain parenchymal fraction</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>0.7449 ± 0.007471</td>
<td>0.7541 ± 0.01085</td>
<td>0.027</td>
</tr>
<tr>
<td>SD</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lesion volume</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean (mm$^3$)</td>
<td>2528 ± 4239</td>
<td>3958 ± 6723</td>
<td>0.637</td>
</tr>
<tr>
<td>SD</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

SD: Standard Deviation

3.3.3 ROI analysis

Brain volumes in ROI were compared between participants with persistent compared to resolved anosmia. The full measurements per ROI can be seen in Table 4.

Significant difference in ROI volume between participants with recovered versus resolved anosmia were detected the right lateral ventricle (14532.4mm$^3$ ±6008.6 vs
9148.2mm³ ±2848.2, p=0.023) and the left ventricle (15087.5mm³ ±6205.1 vs 9512.5mm³ ±2466.5, p=0.019).

3.3.3.1 Persistent anosmia, recovered anosmia and healthy controls – whole brain analysis
Regression analyses revealed no significant differences between volumetric measurements between the three groups of participants. Brain parenchymal fraction measurements were lower in participants with persistent anosmia compared to participants from the other two groups (recovered anosmia and healthy control group) but this difference did not reach statistical significance (p=0.057).

3.3.3.2 ROI analysis: Persistent anosmia, recovered anosmia and healthy controls
ANOVA was carried out in order to identify potential differences in the selected ROI between the three study groups. Table 3.4 summarises the volumetric results from the investigated ROI between the 3 study groups. Left lateral ventricle volume was significantly increased in the persistent anosmia group compared to both other groups (p=0.035).
Table 3.4: Volumetric results from ROI analysis from the three study groups

<table>
<thead>
<tr>
<th>ROI</th>
<th>Group 1 (persistent anosmia)</th>
<th>Group 2 (recovered anosmia)</th>
<th>Group 3 (healthy control group)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Right amygdala (mm³)</td>
<td>931.9 ±104.1</td>
<td>944.9 ±92.4</td>
<td>971.7 ±133.6</td>
<td>0.742</td>
</tr>
<tr>
<td>Left amygdala (mm³)</td>
<td>971.1 ±91.2</td>
<td>986.6 ±108.0</td>
<td>1022.7 ±122.4</td>
<td>0.588</td>
</tr>
<tr>
<td>Brainstem (mm³)</td>
<td>8962.5 ±445.2</td>
<td>9496.6 ±825.2</td>
<td>9845.0 ±1058.3</td>
<td>0.105</td>
</tr>
<tr>
<td>Right hippocampus (mm³)</td>
<td>4650.9 ±319.3</td>
<td>4663.9 ±371.3</td>
<td>4729.5 ±549.8</td>
<td>0.915</td>
</tr>
<tr>
<td>Left hippocampus (mm³)</td>
<td>4449.9 ±288.2</td>
<td>4483 ±388.9</td>
<td>4491 ±408.5</td>
<td>0.959</td>
</tr>
<tr>
<td>Right lateral ventricle (mm³)</td>
<td>14532.4 ±6008.6</td>
<td>9148.2 ±2848.2</td>
<td>10957.2 ±5106.9</td>
<td>0.072</td>
</tr>
<tr>
<td>Left lateral ventricle (mm³)</td>
<td>15087.5 ±6205.1</td>
<td>9512.5 ±2466.5</td>
<td>12008.8 ±3726.9</td>
<td>0.035</td>
</tr>
<tr>
<td>Right anterior insula (mm³)</td>
<td>3985.9 ±447.6</td>
<td>3915.6 ±516.4</td>
<td>4200.3 ±549.8</td>
<td>0.446</td>
</tr>
<tr>
<td>Left anterior insula (mm³)</td>
<td>4224.4 ±486.0</td>
<td>4069.3 ±532.2</td>
<td>4385.4 ±662.7</td>
<td>0.472</td>
</tr>
<tr>
<td>Right inferior temporal gyrus (mm³)</td>
<td>11387.6 ±1311.8</td>
<td>10996.0 ±733.1</td>
<td>11469.6 ±1378.8</td>
<td>0.635</td>
</tr>
<tr>
<td>Left inferior temporal gyrus (mm³)</td>
<td>10743.9 ±820.4</td>
<td>10553.0 ±515.9</td>
<td>11091.4 ±1380.0</td>
<td>0.476</td>
</tr>
<tr>
<td>Right parahippocampal gyrus (mm³)</td>
<td>3008.5 ±392.6</td>
<td>3082.0 ±345.5</td>
<td>3241.0 ±247.1</td>
<td>0.333</td>
</tr>
<tr>
<td>Left parahippocampal gyrus (mm³)</td>
<td>3179.5 ±283.0</td>
<td>3427.5 ±281.7</td>
<td>3446.6 ±307.9</td>
<td>0.128</td>
</tr>
<tr>
<td>Right Posterior insula (mm³)</td>
<td>2357.4 ±235.9</td>
<td>2264.8 ±291.0</td>
<td>2428.8 ±404.2</td>
<td>0.535</td>
</tr>
<tr>
<td>Left posterior insula (mm³)</td>
<td>2308.6 ±244.8</td>
<td>2358.3 ±280.3</td>
<td>2476.5 ±396.9</td>
<td>0.518</td>
</tr>
</tbody>
</table>

ROI: Region of Interest
3.3.3.3 qMRI: Persistent anosmia vs recovered anosmia

For this part of the analysis, 8 participants were included in the persistent anosmia group and 9 participants in the recovered anosmia group. The participants were comparable in terms of age (52.3 ±12.2 vs. 49.7 ±8.3, p=0.672) and sex (88.8% female vs 87.5% female, p>0.999). qMRI measurements were obtained for 10 different imaging modalities for CGM, WM, DGM, brainstem and lesions. The full results of the analyses can be seen in Tables 3.5-3.13.

T1-weighted imaging detected significant differences in CGM (1.481ms ±0.041 vs. 1.430ms ±0.052, p=0.040) as well as DGM (1.274ms ±0.053 vs. 1.221 ±0.035, p=0.035) between participants with persistent versus resolved anosmia. No significant differences were detected in WM, BS or lesions between the two groups (Table 3.5).

Table 3.5: T1 imaging parameters in participant with persistent versus recovered anosmia

<table>
<thead>
<tr>
<th></th>
<th>Persistent anosmia (n=8)</th>
<th>Recovered anosmia (n=9)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>CGM (ms)</td>
<td>1.481 (±0.041)</td>
<td>1.430 (±0.052)</td>
<td>0.040</td>
</tr>
<tr>
<td>WM (ms)</td>
<td>0.960 (±0.413)</td>
<td>0.936 (±0.413)</td>
<td>0.182</td>
</tr>
<tr>
<td>DGM (ms)</td>
<td>1.274 (±0.053)</td>
<td>1.221 (±0.035)</td>
<td>0.035</td>
</tr>
<tr>
<td>Brainstem (ms)</td>
<td>1.252 (±0.751)</td>
<td>1.225 (±0.602)</td>
<td>0.429</td>
</tr>
<tr>
<td>Lesions</td>
<td>1.140 (±0.159)</td>
<td>1.133 (±0.856)</td>
<td>0.928</td>
</tr>
</tbody>
</table>


Comparison of bound pool fraction measurements detected significant differences in DGM between participants with persistent and recovered anosmia (0.075ms ±0.004 vs. 0.080 ±0.004, p=0.028). No significant differences were detected in WM or any of the other parameters (Table 3.6).
Table 3.6: Bound pool fraction parameters in participants with persistent versus recovered anosmia

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Persistent anosmia (n=8)</th>
<th>Recovered anosmia (n=9)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>CGM (ms)</td>
<td>0.062 (±0.003)</td>
<td>0.065 (±0.004)</td>
<td>0.200</td>
</tr>
<tr>
<td>WM (ms)</td>
<td>0.113 (±0.006)</td>
<td>0.118 (±0.005)</td>
<td>0.078</td>
</tr>
<tr>
<td>DGM (ms)</td>
<td>0.075 (±0.004)</td>
<td>0.080 (±0.004)</td>
<td>0.028</td>
</tr>
<tr>
<td>Brainstem (ms)</td>
<td>0.081 (±0.010)</td>
<td>0.087 (±0.007)</td>
<td>0.177</td>
</tr>
<tr>
<td>Lesions</td>
<td>0.093 (±0.017)</td>
<td>0.088 (±0.010)</td>
<td>0.536</td>
</tr>
</tbody>
</table>


T2b and T2 imaging analysis showed no statistically significant differences between participants with persistent and recovered anosmia (Table 3.7 and Table 3.8).

Table 3.7: T2b parameters in participant with persistent versus recovered anosmia

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Persistent anosmia (n=8)</th>
<th>Recovered anosmia (n=9)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>CGM (ms)</td>
<td>9.799 (±0.137)</td>
<td>9.789 (±0.249)</td>
<td>0.914</td>
</tr>
<tr>
<td>WM (ms)</td>
<td>10.39 (±0.175)</td>
<td>10.24 (±0.236)</td>
<td>0.144</td>
</tr>
<tr>
<td>DGM (ms)</td>
<td>9.507 (±0.130)</td>
<td>9.432 (±0.250)</td>
<td>0.444</td>
</tr>
<tr>
<td>Brainstem (ms)</td>
<td>10.82 (±0.252)</td>
<td>10.63 (±0.273)</td>
<td>0.149</td>
</tr>
<tr>
<td>Lesions</td>
<td>9.774 (±0.690)</td>
<td>10.60 (±1.478)</td>
<td>0.215</td>
</tr>
</tbody>
</table>


Table 3.8: T2 imaging parameters in participant with persistent versus recovered anosmia

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Persistent anosmia (n=8)</th>
<th>Recovered anosmia (n=9)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>CGM (ms)</td>
<td>0.071 (±0.004)</td>
<td>0.070 (±0.005)</td>
<td>0.663</td>
</tr>
<tr>
<td>WM (ms)</td>
<td>0.062 (±0.003)</td>
<td>0.063 (±0.002)</td>
<td>0.589</td>
</tr>
<tr>
<td>DGM (ms)</td>
<td>0.060 (±0.005)</td>
<td>0.058 (±0.003)</td>
<td>0.368</td>
</tr>
<tr>
<td>Brainstem (ms)</td>
<td>0.070 (±0.005)</td>
<td>0.072 (±0.006)</td>
<td>0.391</td>
</tr>
<tr>
<td>Lesions</td>
<td>0.087 (±0.011)</td>
<td>0.078 (±0.010)</td>
<td>0.147</td>
</tr>
</tbody>
</table>

Diffusion imaging analysis showed no significant between participants with persistent and recovered anosmia, in FA nor MD (Table 3.9 and Table 3.10).

**Table 3.9: Fractional anisotropy in participant with persistent versus recovered anosmia**

<table>
<thead>
<tr>
<th></th>
<th>Persistent anosmia (n=8)</th>
<th>Recovered anosmia (n=9)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>CGM (ms)</td>
<td>0.155 (±0.007)</td>
<td>0.159 (±0.007)</td>
<td>0.242</td>
</tr>
<tr>
<td>WM (ms)</td>
<td>0.385 (±0.021)</td>
<td>0.396 (±0.013)</td>
<td>0.247</td>
</tr>
<tr>
<td>DGM (ms)</td>
<td>0.225 (±0.014)</td>
<td>0.228 (±0.150)</td>
<td>0.633</td>
</tr>
<tr>
<td>Brainstem (ms)</td>
<td>0.408 (±0.010)</td>
<td>0.418 (±0.021)</td>
<td>0.431</td>
</tr>
<tr>
<td>Lesions</td>
<td>0.278 (±0.084)</td>
<td>0.310 (±0.063)</td>
<td>0.424</td>
</tr>
</tbody>
</table>


**Table 3.10: Mean diffusivity in participant with persistent versus recovered anosmia**

<table>
<thead>
<tr>
<th></th>
<th>Persistent anosmia (n=8)</th>
<th>Recovered anosmia (n=9)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>CGM (ms)</td>
<td>0.00097 (±2.676e-005)</td>
<td>0.00094 (±4.857e-005)</td>
<td>0.139</td>
</tr>
<tr>
<td>WM (ms)</td>
<td>0.00083 (±3.541e-005)</td>
<td>0.00081 (±1.300e-005)</td>
<td>0.105</td>
</tr>
<tr>
<td>DGM (ms)</td>
<td>0.00095 (±5.257e-005)</td>
<td>0.00092 (±3.452e-005)</td>
<td>0.071</td>
</tr>
<tr>
<td>Brainstem (ms)</td>
<td>0.00092 (±3.745e-005)</td>
<td>0.00090 (±3.820e-005)</td>
<td>0.550</td>
</tr>
<tr>
<td>Lesions</td>
<td>0.00117 (±0.00012)</td>
<td>0.00107 (±8.904e-005)</td>
<td>0.081</td>
</tr>
</tbody>
</table>


Intracellular volume fraction and extracellular volume fraction were comparable between participants with persistent versus recovered anosmia (Table 3.11 and Table 3.12).
Table 3.11: Intracellular volume fraction in participant with persistent versus recovered anosmia

<table>
<thead>
<tr>
<th></th>
<th>Persistent anosmia (n=8)</th>
<th>Recovered anosmia (n=9)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>CGM (ms)</td>
<td>0.355 (±0.015)</td>
<td>0.365 (±0.019)</td>
<td>0.251</td>
</tr>
<tr>
<td>WM (ms)</td>
<td>0.490 (±0.030)</td>
<td>0.503 (±0.011)</td>
<td>0.256</td>
</tr>
<tr>
<td>DGM (ms)</td>
<td>0.385 (±0.028)</td>
<td>0.397 (±0.015)</td>
<td>0.289</td>
</tr>
<tr>
<td>Brainstem (ms)</td>
<td>0.486 (±0.016)</td>
<td>0.494 (±0.028)</td>
<td>0.488</td>
</tr>
<tr>
<td>Lesions</td>
<td>0.300 (±0.053)</td>
<td>0.344 (±0.061)</td>
<td>0.193</td>
</tr>
</tbody>
</table>


Table 3.12: Extracellular volume fraction in participant with persistent versus recovered anosmia

<table>
<thead>
<tr>
<th></th>
<th>Persistent anosmia (n=8)</th>
<th>Recovered anosmia (n=9)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>CGM (ms)</td>
<td>0.528 (±0.013)</td>
<td>0.535 (±0.013)</td>
<td>0.258</td>
</tr>
<tr>
<td>WM (ms)</td>
<td>0.353 (±0.026)</td>
<td>0.357 (±0.021)</td>
<td>0.729</td>
</tr>
<tr>
<td>DGM (ms)</td>
<td>0.469 (±0.029)</td>
<td>0.483 (±0.029)</td>
<td>0.316</td>
</tr>
<tr>
<td>Brainstem (ms)</td>
<td>0.296 (±0.019)</td>
<td>0.301 (±0.022)</td>
<td>0.592</td>
</tr>
<tr>
<td>Lesions</td>
<td>0.421 (±0.062)</td>
<td>0.447 (±0.091)</td>
<td>0.543</td>
</tr>
</tbody>
</table>


ODI analysis revealed a significant difference between participants with persistent compared to recovered anosmia (p=0.031)(Table 3.13).

Table 3.13: ODI in participant with persistent versus recovered anosmia

<table>
<thead>
<tr>
<th></th>
<th>Persistent anosmia (n=8)</th>
<th>Recovered anosmia (n=9)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>CGM (ms)</td>
<td>0.484 (±0.004)</td>
<td>0.478 (±0.005)</td>
<td>0.031</td>
</tr>
<tr>
<td>WM (ms)</td>
<td>0.256 (±0.007)</td>
<td>0.253 (±0.009)</td>
<td>0.412</td>
</tr>
<tr>
<td>DGM (ms)</td>
<td>0.406 (±0.020)</td>
<td>0.410 (±0.020)</td>
<td>0.736</td>
</tr>
<tr>
<td>Brainstem (ms)</td>
<td>0.256 (±0.010)</td>
<td>0.251 (±0.008)</td>
<td>0.282</td>
</tr>
<tr>
<td>Lesions</td>
<td>0.261 (±0.089)</td>
<td>0.238 (±0.060)</td>
<td>0.580</td>
</tr>
</tbody>
</table>

There were no significant differences in VFISO between participants with persistent and recovered anosmia (Table 3.14).

**Table 3.14: Isotropic diffusion volume fraction (VFISO) in participant with persistent versus recovered anosmia**

<table>
<thead>
<tr>
<th></th>
<th>Persistent anosmia (n=8)</th>
<th>Recovered anosmia (n=9)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>CGM (ms)</td>
<td>0.117 (±0.012)</td>
<td>0.099 (±0.024)</td>
<td>0.091</td>
</tr>
<tr>
<td>WM (ms)</td>
<td>0.157 (±0.018)</td>
<td>0.140 (±0.015)</td>
<td>0.060</td>
</tr>
<tr>
<td>DGM (ms)</td>
<td>0.146 (±0.028)</td>
<td>0.120 (±0.029)</td>
<td>0.080</td>
</tr>
<tr>
<td>Brainstem (ms)</td>
<td>0.218 (±0.026)</td>
<td>0.204 (±0.029)</td>
<td>0.336</td>
</tr>
<tr>
<td>Lesions</td>
<td>0.278 (±0.071)</td>
<td>0.210 (±0.054)</td>
<td>0.067</td>
</tr>
</tbody>
</table>


### 3.3.4 qMRI: Persistent anosmia versus recovered anosmia vs healthy control group

Regression analysis using a general linear model with age and sex as confounders was used to compare results between the 3 study groups, participants with persistent anosmia, participants with recovered anosmia and the SARS-CoV-2 negative healthy control group.

Significant results were identified in the following areas:

- **T2b Imaging: CGM**

  A general linear model revealed a significant group effect (p=0.009) in a model including age and sex as co-variates. Although there were differences observed between both the persistent anosmia group (p=0.004) compared to the healthy control group a significant age interaction was also seen (p=0.020). Parameter estimates from the linear regression can be seen in Table 3.15.
Table 3.15: Linear regression analysis of T2b imaging

<table>
<thead>
<tr>
<th></th>
<th>Beta</th>
<th>95% CI lower</th>
<th>95% CI upper</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>0.008</td>
<td>0.001</td>
<td>0.014</td>
<td>0.020</td>
</tr>
<tr>
<td>Sex</td>
<td>-0.034</td>
<td>-0.218</td>
<td>0.150</td>
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</tr>
<tr>
<td>Persistent anosmia</td>
<td>0.008</td>
<td>0.077</td>
<td>-0.426</td>
<td>0.007</td>
</tr>
<tr>
<td>(Group 1)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Recovered anosmia</td>
<td>-0.792</td>
<td>-1.153</td>
<td>-0.430</td>
<td>0.895</td>
</tr>
<tr>
<td>(Group 2)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

CI: Confidence Interval

- **T2b imaging: Brainstem**
  
  A general linear model revealed a significant group effect (p<0.001) in a model including age and sex as covariates. Significant differences were identified between the persistent anosmia group compared to the healthy control group. Parameter estimated from the linear regression can be seen in table 3.16.

Table 3.16: Linear regression analysis of T2b imaging

<table>
<thead>
<tr>
<th></th>
<th>Beta</th>
<th>95% CI lower</th>
<th>95% CI upper</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>0.002</td>
<td>-0.09</td>
<td>0.013</td>
<td>0.705</td>
</tr>
<tr>
<td>Sex</td>
<td>0.367</td>
<td>0.044</td>
<td>0.690</td>
<td>0.030</td>
</tr>
<tr>
<td>Persistent anosmia</td>
<td>-0.596</td>
<td>-0.953</td>
<td>-0.239</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>(Group 1)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Recovered anosmia</td>
<td>-0.792</td>
<td>-1.153</td>
<td>-0.430</td>
<td>0.208</td>
</tr>
<tr>
<td>(Group 2)</td>
<td></td>
<td></td>
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<td></td>
</tr>
</tbody>
</table>

CI: Confidence Interval

- **VFISO: CGM**

  A general linear model revealed a significant group effect (p=0.001) in a model including age and sex covariates. However no significant differences were seen in the between group analyses. Parameter estimates are displayed in Table 3.17.
Table 3.17: Linear regression analysis of VFISO in CGM

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Beta</th>
<th>95% CI lower</th>
<th>95% CI upper</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>0.001</td>
<td>0.000</td>
<td>0.001</td>
<td>0.097</td>
</tr>
<tr>
<td>Sex</td>
<td>-0.002</td>
<td>-0.022</td>
<td>0.019</td>
<td>0.864</td>
</tr>
<tr>
<td>Persistent anosmia (Group 1)</td>
<td>0.002</td>
<td>-0.018</td>
<td>0.021</td>
<td>0.866</td>
</tr>
<tr>
<td>Recovered anosmia (Group 2)</td>
<td>0.015</td>
<td>-0.004</td>
<td>0.035</td>
<td>0.118</td>
</tr>
</tbody>
</table>

CI: Confidence Interval. VFISO: Isotropic diffusion volume fraction. CGM: Cortical Grey Matter

VFISO: Lesions

A general linear model revealed a significant group effect in VFISO for lesions (p=0.014) in a model including age and sex covariates. VFISO in the lesions was higher in the recovered anosmia group compared to the healthy control group. Parameter estimates can be seen in Table 3.18

Table 3.18: Linear regression analysis of in lesions

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Beta</th>
<th>95% CI lower</th>
<th>95% CI upper</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>0.000</td>
<td>-0.002</td>
<td>0.003</td>
<td>0.519</td>
</tr>
<tr>
<td>Sex</td>
<td>-0.134</td>
<td>-0.204</td>
<td>-0.063</td>
<td>0.001</td>
</tr>
<tr>
<td>Persistent anosmia (Group 1)</td>
<td>0.007</td>
<td>-0.059</td>
<td>0.070</td>
<td>0.822</td>
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<td>Recovered anosmia (Group 2)</td>
<td>0.086</td>
<td>0.025</td>
<td>0.147</td>
<td>0.008</td>
</tr>
</tbody>
</table>

CI: Confidence Interval. VFISO: Isotropic diffusion volume fraction.

3.4 Discussion

The FORECAST MRI sub-study followed up a subset of participants from the FORECAST Study, who had objective olfactory function evaluation through UPSIT testing. MRI brain imaging was performed with a detailed imaging protocol utilising both qualitative and qualitative MRI, aiming to investigate the presence of CNS pathology in people with persistent and recovered anosmia following COVID-19. MRI parameters were
initially compared between participants from the FORECAST study with persistent anosmia compared to participants with recovered anosmia. Subsequently, parameters from participants in these two groups were also compared with an age- and sex-matched control group. The results presented here are preliminary and exploratory results from the FORECAST MRI sub-study.

Whole brain volumetric analyses between participants with persistent and recovered anosmia revealed comparable CGM and DGM volumes, suggesting that the differences seen in the qMRI analysis present micro-structural abnormalities. Differences were however noted in brain parenchymal fraction volume which was lower in participants with persistent compared to resolved anosmia. Brain parenchymal fraction is a marker of atrophy, commonly used in the monitoring of neurodegenerative disorders[25]. A change in brain parenchymal fraction has not previously been reported following COVID-19, with the exception of atrophy of the olfactory bulb observed in people with anosmia[8]. As segmentation of the olfactory bulb and other relevant areas of the olfactory tract as ROI have not yet been performed in our data, it remains unclear whether this is a finding related to atrophy along the olfactory tract in the CNS due to COVID-19. Therefore, the significance of this finding in the context of this study and the small population remains uncertain, however certainly requires further investigation with larger sample sizes to determine if there is a relationship between COVID-19 and increased cerebral atrophy, as this would be a significant finding with considerable clinical implications.

The ROIs investigated to date do not yet include all relevant areas with regard to the olfactory tract as segmentation of these remains ongoing. The difference observed in the lateral ventricle between people with persistent compared to resolved anosmia may be of significance in this context. A rostral migratory stream in the adult human brain has been demonstrated and localised around the lateral ventricle[26]. From there, progenitor cells migrate to the olfactory bulb and develop into mature neurons[26]. A larger lateral ventricle size has previously been described in patients with anosmia due to different pathologies[27]. It is therefore plausible that this difference in lateral ventricle volume reflects a difference in neuronal regeneration
between people who did compared to those who did not regain their olfactory function following COVID-19. Interestingly, comparison between the three groups demonstrated that both the groups of participants with persistent and recovered anosmia had larger lateral ventricle volumes compared to the control group, suggesting that this may be linked to processes linked to neurological recovery.

qMRI enables the comparison of physical and chemical variables in tissue composition within the CNS[28]. qMRI parameters have the benefit of being extremely sensitive and are able to detect changes that are preceding or accompanying either volumetric or focal changes, which are not visible using qualitative clinical MRI imaging[28]. However, the limitation of this extreme sensitivity can be poor specificity, as well as susceptibility to age-related changes[29]. Therefore, for this preliminary data analysis of this study, groups were age- and sex-matched. The automated quantitative processing of the MRI data performed in this study enables us to detect subtle microstructural changes within the CNS that would not be visible at the level of a clinical qualitative scan, which in the context of the setting of this study point toward a neuroinflammatory disease process induced by SARS-CoV-2 neurotropism.

T1 refers to the longitudinal relaxation time, and is a fundamental qMRI parameter used widely for the characterisation of healthy and pathological tissue[30]. When comparing T1 across the whole brain in people with persistent versus recovered anosmia, higher T1 values were seen in both cortical and deep grey matter in participants with persistent anosmia. No differences were seen in WM between the groups on T1. This finding suggests the presence of an inflammatory process within grey matter in people with persistent compared to resolved anosmia[17].

Bound pool fraction , also known as the macromolecular proton fraction or pool size ratio, is a key biophysical parameter for the quantitative description of the magnetization transfer effect in biological tissues and conveys information about their macromolecular content[23]. Bound pool fraction is a marker of myelin integrity, which is independent of axonal loss[23]. We observed a reduction in bound pool fraction in DGM in participants with persistent compared to resolved anosmia. The ability of SARS-
CoV-2 to affect myelin has been previously described in a number of clinical imaging studies, illustrating demyelinating lesions with radiological appearances akin to multiple sclerosis[31-33]. Proposed mechanisms include a reduction in populations of CD4 + T cells, CD8 + T cells, B cells, and NK cells; pro-inflammatory cytokine over-secretion, contributing to a pro-inflammatory state which may in turn trigger autoimmune reactions[34].

When comparing participants with persistent versus resolved anosmia, no significant differences were seen in T2 or T2b imaging parameters. T2 refers to the transverse relaxation time and is the time constant which determines the rate at which excited protons reach equilibrium[35]. T2 provides structural information and includes information on water content[35]. No changes were seen between participants with persistent versus recovered anosmia from acquired diffusion data. Diffusion MRI provides an indirect measure of size, orientation and shape of cellular structures, providing unique microstructural information non-invasively[36]. Diffusion tensor imaging (DTI) is a methodology used to calculate diffusion indices such as fractional anisotropy and mean diffusivity, which are markers of neuroinflammation. Mean diffusivity in DGM was higher in participants with persistent anosmia, but this did not reach statistical significance (p=0.070).

Neurite orientation dispersion and density imaging (NODDI) is a diffusion MRI modality, more specific than DTI in capturing the microstructural substrates, possibly underpinning neurodegeneration[37,38]. Intracellular volume fraction (a measure of intracellular space and a marker of axonal mass) and extracellular volume fraction (a marker of soma mass) analysis showed no differences in measured parameters between participants in the persistent versus recovered anosmia groups[37]. ODI, which refers to the orientation of structures[37], was significantly higher in participants with persistent compared to recovered anosmia (p=0.031). Taken together, the findings comparing participants with persistent versus resolved anosmia suggest the presence of inflammation, in both cortical and deep grey matter in participants with persistent anosmia, compared to participants whose smell function fully returned following COVID-19.
Subsequently, we compared the same qMRI data parameters, using regression analyses between three study groups: participants with persistent anosmia, participants with recovered anosmia and an age and sex matched control group, who had no symptoms during the COVID-19 pandemic with confirmed negative SARS-CoV-2 antibody status. The first notable finding in this analysis was the significant differences detected in T2b between the persistent anosmia groups, compared to the control group in CGM and the brainstem. Mechanisms affecting T2b would either imply an increase in cellularity, which is unlikely in the case of anosmia, or alternatively a change in susceptibility, which can occur as a result of iron deposition. It is now established that SARS-COV-2 results in altered iron metabolism. SARS-CoV-2 has a high resemblance to hepcidin, a key regulator of iron metabolism in humans[39]. This hepcidin-like activity of the virus may be driving the iron dysmetabolism seen in COVID-19, potentially driving ferritin accumulation and ferroptosis[40]. This altered iron homeostasis and the resulting imbalance between bound and free iron, may be sufficient to alter the local relaxation properties of neural tissue in the CNS, which poses a potential explanation for the shortened T2b observed in this study. With regard to the brainstem findings, olfactory receptors are present in the brainstem, although their role remains unclear, as the olfactory nerve does not attach to the brainstem[41]. Gustatory pathways do however pass via the brainstem[42]. The significance of this finding remains uncertain. However, although no objective evaluation of gustatory function was carried out in these participants, it is plausible that this is an abnormality that could relate to gustatory pathways being affected by SARS-CoV-2.

Finally, significant group effects were seen in VFISO, which indicates the amount of mobile water in the CNS, both within and without the CSF, in CGM as well as within brain lesions. The between group comparisons did not show any significant differences between groups in CGM, likely due to the small sample sizes. The lesions’ VFISO was higher in participants with recovered anosmia compared to the control group. Altered VFISO within lesions suggests an inflammatory processes, although establishing the relevance of this finding with regard to recovery of anosmia will require larger sample sizes and longitudinal imaging[37,38].
The preliminary and exploratory results of this study are in line with the published literature, suggesting SARS-CoV-2 causes subtle microstructural grey matter changes[14,15]. The study by Douaud et al., demonstrated a significant impact of COVID-19 on both olfactory and gustatory cortical areas, resulting in loss of grey matter in these areas. It is however not known what percentage of the participants in this study experienced smell and taste symptoms during the acute illness and whether this persisted at the time imaging was carried out[15]. This study is the first to our knowledge to directly compare people with persistent versus recovered anosmia due to COVID-19 using quantitative brain imaging. The early results from this study suggest persistent and ongoing neuroinflammation in people with anosmia as a manifestation of long-COVID. Although it is not yet possible to determine the chronicity of these changes, these findings suggest that unresolving anosmia following the acute phase of the infection may be a clinical indicator of ongoing neuroinflammation. If the growing body of evidence regarding the neurotropism of SARS-CoV-2 confirms ongoing inflammatory changes in this group of patients, clinical trials using pharmacological agents proven to be effective against neuroinflammation in other clinical contexts will be warranted for this patient population. Data from studies investigating the long-term effects of COVID-19 are demonstrating the potent ability of SARS-CoV-2 to generate both systemic and organ-specific inflammation and support the need for more clinical trials evaluating the use of immune-modulating agents in the treatment of COVID-19[43,44]. Furthermore, utilising the risk factors for ongoing chemosensory dysfunction identified in Chapter 2 could enable identifying people at the highest risk of persistent symptoms, and consequentially neuroinflammation and enable prioritising them for treatment.

3.5 Future work

The results presented in this study are preliminary and exploratory and further work will be required in order to validate the presented results and hypotheses. Processing and analyses of acquired data are ongoing and future work within this study will include further segmentation of ROI including the full areas comprising the olfactory and
gustatory cortex, including the olfactory bulb. Furthermore, in order to address the question of whether the changes in the olfactory network occur exclusively in people who developed olfactory symptoms, a cohort of participants who recovered from COVID-19 and did not have a loss of their sense of smell have been recruited. In addition, functional MRI data and perfusion imaging were also carried out in the existing cohort and are currently being processed. Furthermore, given the majority of participants in the current studies are over the age of 50, we recruited a cohort of young participants with recovered anosmia, to investigate whether age has an effect on the susceptibility to the neurotropism of SARS-CoV-2. Beyond the work carried out of in this study, larger cohorts of participants as well as longitudinal imaging will be required in order to determine both the extent of these neuroinflammatory manifestations of COVID-19, but also their chronicity, reversibility and responsiveness to treatment.

3.6 Conclusions

Early data from this study provide preliminary evidence of neuroinflammation and grey matter microstructural pathology in patients with persistent anosmia following COVID-19. These findings combined with other recently published work, provide evidence for the neuroinvasivity of SARS-CoV-2 via the olfactory pathway. While the permanency of these findings, the likelihood of recovery, as well the long-term consequences of these pathological processes remain unknown, this highlights the importance of being able to identify at-risk patient populations for follow up and potential treatments once these become available.
3.7 Chapter 3 References


4. Chapter 4: Chemosensation of dietary fat in people with obesity and its relationship to GDF15 secretion

4.1 Introduction

The role of taste signalling in perception of energy-dense foods as highly palatable has attracted a growing scientific interest in recent years. The ability of humans to taste fat independent of sweet, salty, bitter, sour and umami, as well as textural and olfactory cues has been well described[1,2]. Taste is a highly dynamic function and adapts according to exposure. Taste adaptation, a transient reduction in sensitivity following continuous exposure, occurs with sweet and fatty stimuli in individuals with healthy weight and obesity[3,4]. Taste sensitivity to fatty and sweet stimuli reduces with weight gain and reduced taste sensitivity to sweet stimuli and dietary fat is observed in people with obesity[2,5,6]. Interestingly, taste buds able to detect fatty acids express GLP-1, ghrelin and CCK[7-9]. At a cellular level, the fatty acid oleic acid (C18:1) has been shown to directly stimulate GLP-1 production[10].

Furthermore, it has been suggested that reduced fatty acid detection in the oral cavity and the GI tract leads to impaired satiety[11]. In a study by Keast et al., healthy participants were divided into hyposensitive and hypersensitive based on their ability to taste fatty acids, using an oleic acid fatty acid taste detection test[11]. Participants subsequently consumed a high-fat breakfast followed by an ad libitum buffet lunch later on the same day and hyposensitive participants were shown to consume excess calories compared to hypersensitive participants, which supports the notion that impaired fatty acid chemosensation leads to reduced satiety and excess calorie intake from dietary fat[11]. Furthermore, Stewart et al., demonstrated that high taste sensitivity to fatty acids is associated with lower energy intake and lower BMI, which also suggests a role for tasting fat in body weight regulation[12].

Taste following bariatric surgery changes both in terms of an increased taste sensitivity, but also in terms of foods reportedly tasting different and patients commonly
experience altered food preferences across all food categories[13,14]. Energy-dense foods, in particular foods with a high fat content are also commonly perceived as aversive[15,16]. We have previously demonstrated that changes in taste following bariatric surgery are a predictor for a higher post-operative weight loss[17]. Understanding the association between orosensory detection of fat, the dietary preference for fat and the physiological drivers for the taste changes following bariatric surgery could form a basis for the development of novel therapeutic strategies for obesity.

Data are now also emerging from animal studies with regard to the role of GDF15 in food preference for dietary fat. GDF15, a pro-inflammatory cytokine, is known to suppress appetite through non-homeostatic CNS pathways via the area postrema[18]. Marked changes in food preference have been observed in animals following GDF15 administration. When presented with a high- versus low-fat meal option, both mice and non-human primates that originally preferred a high-fat feed switched their preference to the low-fat feed whilst receiving GDF15[19]. In a more recent study using a diet-induced obesity mouse model, GDF15 administration was shown to consistently reduce preference for fat, whereas it did not affect preference for protein or carbohydrates[20]. Furthermore, acute administration of GDF15 to rodents has been shown to induce conditioned taste aversion[21]. Taken together, these findings suggest a role for GDF15 for driving taste preference away from high-fat and towards low-fat diets, highlighting the high potential for GDF15 pharmacotherapy as a therapeutic strategy for obesity.

A small number of studies to date, have investigated the effect of bariatric surgery on circulating GDF15 levels. Both RYGB and SG have been associated with elevations in GDF15 following surgery[22-25]. These findings however need to be interpreted with caution, in light of large variations in post-operative GDF15 levels between participants in these studies, until more data are available to explain the relationship between post-bariatric surgery weight loss and GDF15 secretion. The role of GDF15 as a potential driver of the shift in food preference away from high-fat foods following bariatric surgery has not yet been investigated. However, a number of studies investigating
changes in GDF15 in response to a glucose load or to a meal have shown no significant acute changes in circulating GDF15 levels[26,27]. In addition, GDF15 is known to be present in saliva, but the relationship between taste signaling, nutrient ingestion and salivary GDF15 secretion has not yet been investigated[28].

Therefore, the work in this chapter presents the results from four studies, which aimed to investigate:

1. The relationship between GDF15 levels in blood and saliva in people with healthy weight in the fasted state and following a meal (Study 1).
2. The effect of a meal on circulating GDF15 levels in people with obesity (Study 2).
3. The relationship between GDF15 levels in blood and saliva in people with obesity (Study 3).
4. Taste sensitivity to dietary fat (oleic acid) in people with severe obesity (Study 3).
5. The response of salivary and circulating GDF15 to dietary fat in people with severe obesity (Study 3).
6. The differences in taste sensitivity and preference for dietary fat in people with severe obesity compared to people who have undergone bariatric surgery (Study 3).
7. The effect of RYGB and SG on GDF15 secretion profiles in people with and without T2DM and their relationship to taste, food preference and weight loss (Study 4).

4.2 Study 1: Investigating the relationship between salivary and circulating GDF15 in response to the taste of food and a meal

4.2.1 Hypotheses

1. Salivary and circulating GDF15 follow independent secretion profiles.
2. Salivary GDF15 levels are altered in response to fasting, tasting food and/or eating.
4.2.2 Methods

4.2.2.1 Participants
Healthy adult volunteers were invited to take part in this study. Participants were recruited through internal advertisement at UCL. The study received UCL Ethics Committee approval (13097/001). The inclusion criteria were adult male and female participants aged 18 years and over with a good understanding of written and spoken English. The study’s exclusion criteria were a history of any chronic medical condition, food allergies and smoking. Participants were recruited until clinical studies were paused due to the COVID-19 pandemic. As the aim of this study was to investigate trends in adults of healthy weight, this study’s results were treated as exploratory.

4.2.2.2 Study assessments
Participants arrived in the morning, following an overnight fast. Participants were instructed to avoid alcohol consumption and strenuous physical activity for 24 hours prior to the study visit, but otherwise follow their usual diet and exercise routine. Written informed consent was obtained prior to any study procedures being undertaken. An intravenous cannula was inserted into a vein in the participant’s arm and they were allowed to rest for 45 minutes, in order to avoid the stress of cannulation impacting upon results[29]. Participants’ height was recorded using a stadiometer. Body composition analysis was performed using bioelectrical impedance analysis (BIA) (Tanita DC-430 MA S, Manchester, UK), measuring body weight, body fat, muscle and bone mass. BMI was calculated using the height and weight measurements obtained at the study visit.

Following a 45 minute acclimatisation period, paired fasted blood and saliva samples were collected (T0 and S0). Participants were then given a standardised meal, consisting of 903kcal (Table 4.1). They were instructed to consume this within a 15-minute period. The energy intake content was calculated at the end of the mealtime for participants who were unable to complete the meal. The meal and macronutrient composition of the meal can be seen in Table 1. Participants were given 15 minutes to eat and a paired blood and saliva sample were collected immediately after they
finished eating (T15 and S15). Paired blood and saliva samples were taken at 30-minute intervals until 180 minutes from the meal (30, 60, 90, 120, 150 and 180 minutes).

**Table 4.1: Mixed meal**

<table>
<thead>
<tr>
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<th>Energy</th>
<th>Carbohydrate</th>
<th>Protein</th>
<th>Fat</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cheese sandwich (Sainsbury’s no mayo cheese sandwich – 133g)</td>
<td>397</td>
<td>41.4g</td>
<td>17.6g</td>
<td>17.6g</td>
</tr>
<tr>
<td>Strawberry and banana smoothie (Innocent – 250ml)</td>
<td>134</td>
<td>12g</td>
<td>0.5g</td>
<td>0.0g</td>
</tr>
<tr>
<td>Blueberry muffin (Country Choice – 125g)</td>
<td>372</td>
<td>43.3g</td>
<td>4.8g</td>
<td>19.6g</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>903kcal</td>
<td>96.7</td>
<td>22.9g</td>
<td>37.2g</td>
</tr>
</tbody>
</table>

4.2.2.3 Sample processing

Blood samples for GDF15 were drawn into EDTA tubes primed with aprotinin, a kallikrein inhibitor (aprotinin 10,000 KIU/mL, Nordic Pharma, 5000 units per mL of blood). Collection tubes were immediately transferred into an ice box and centrifuged at 1800 rpm at 4°C for 10 minutes following collection. Plasma was separated and stored in aliquots at -80°C until analysis.

Saliva samples were collected into Saliva Bio collection tubes (Salimetrics) using the passive drooling technique[30]. The collection tubes were primed with aprotinin (aprotinin 10,000 KIU/ml, Nordic Pharma, 5000 units/mL of saliva). Saliva samples were centrifuged at 2500rpm for 10 minutes at 4°C and stored at -80°C until analysis.

GDF15 was measured using a human GDF15 Quantikine enzyme-linked immunosorbent assay (ELISA) assay (R&D systems; sensitivity 0.0-0.4pg/mL; intra-assay variability 1.8% at 238pg/mL, 2.2% at 456pg/mL and 2.8% at 886pg/mL; inter-assay variability 6.0% at 225pg/mL, 4.7% at 442pg/mL and 5.6% at 900pg/mL). All samples from each participant were analysed on the same plate and assay kits with same LOT number were used where possible for the study.
4.2.2.4 Statistical analyses
Statistical analysis was conducted using GraphPad Prism Version 8 and SPSS Version 25 (IBM Statistics). Results are expressed as calculated means with standard deviation (SD). The Gaussian distribution was assessed by D’Agostino-Pearson normality test and parametric (t-tests) or non-parametric tests were used as appropriate. For GDF15 assay results, the integrated area-under-the-curve (AUC) for plasma and salivary levels of GDF15 versus time was calculated using the trapezoid rule. The AUC was also calculated using delta values, representing the change in levels from the baseline fasted sample (T0/S0). Values as means with standard error of the mean (SEM). Comparisons between GDF15 levels at two different time points were undertaken using a t-test or Mann-Whitney test as appropriate. One-way ANOVA repeated measures models were used to analyze repeat GDF15 measurements over time. A correction for multiple comparisons was applied using a Bonferroni correction. Pearson’s correlation analyses were performed to investigate the relationship between GDF15 and parameters of body composition. Statistical significance was assumed at the 0.05 level.

4.2.3 Results

4.2.3.1 Study population
A total of 13 healthy adult volunteers were recruited. The study group’s demographics, BMI and body composition results from the BIA measurements can be seen in Table 4.2. The baseline saliva sample from one participant had to be excluded due to poor quality.
Table 4.2: Study 1 participants’ characteristics

<table>
<thead>
<tr>
<th>Healthy volunteer participant demographics (n=13)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Sex</strong></td>
</tr>
<tr>
<td>69.2% Female (n=9)</td>
</tr>
<tr>
<td>30.8% Male (n=4)</td>
</tr>
<tr>
<td><strong>Age (years)</strong></td>
</tr>
<tr>
<td>24.8 years (±4.9)</td>
</tr>
<tr>
<td><strong>BMI (kg/m²)</strong></td>
</tr>
<tr>
<td>21.7 (±2.1)</td>
</tr>
<tr>
<td><strong>Ethnicity</strong></td>
</tr>
<tr>
<td>White</td>
</tr>
<tr>
<td>30.8% (n=4)</td>
</tr>
<tr>
<td>Mixed/Multiple Ethnicities</td>
</tr>
<tr>
<td>15.4% (n=2)</td>
</tr>
<tr>
<td>Asian/Asian Chinese/Asian Indian</td>
</tr>
<tr>
<td>53.8% (n=7)</td>
</tr>
<tr>
<td><strong>BIA results (n=13)</strong></td>
</tr>
<tr>
<td><strong>Weight (kg)</strong></td>
</tr>
<tr>
<td>58.8 (±8.5)</td>
</tr>
<tr>
<td><strong>Fat %</strong></td>
</tr>
<tr>
<td>19.2 (±7.6)</td>
</tr>
<tr>
<td><strong>Fat mass (kg)</strong></td>
</tr>
<tr>
<td>13.5 (±9.3)</td>
</tr>
<tr>
<td><strong>Fat free mass (FFM) (kg)</strong></td>
</tr>
<tr>
<td>47.9 (±7.8)</td>
</tr>
<tr>
<td><strong>Muscle mass (kg)</strong></td>
</tr>
<tr>
<td>45.1 (±7.3)</td>
</tr>
<tr>
<td><strong>Bone mass (kg)</strong></td>
</tr>
<tr>
<td>2.4 (±0.4)</td>
</tr>
<tr>
<td><strong>Visceral fat rating (scale 1-13)</strong></td>
</tr>
<tr>
<td>1: 69.2% (n=9)</td>
</tr>
<tr>
<td>2: 15.4% (n=2)</td>
</tr>
<tr>
<td>3: 7.7% (n=1)</td>
</tr>
<tr>
<td>4: 7.7% (n=1)</td>
</tr>
</tbody>
</table>

4.2.3.2 Plasma GDF15

The mean fasted GDF15 plasma value in this study cohort was 377.2pg/mL (±132.2).

The mean AUC for GDF15 from 0-180 minutes was 60670pg/mL (±5268, 95% CI 50344 to 70995) and the trend of the plasma levels measured over time can be seen in Figure 4.1A.

The AUC of the change in GDF15 levels from baseline (delta values) was 4122 (±2330, CI 0 to 8700) and a graphical representation of the change from the baseline value over time can be seen in Figure 4.1B.
In order to calculate the effect of tasting the meal on plasma GDF15 levels, the fasted (T0 sample, taken immediately prior to commencing the meal) and the 15-minute sample (taken immediately after completing the meal were compared. The mean 15-minute GDF15 level was 337.9pg/mL (±106.5). This reflected a mean reduction from baseline of -129.3pg/mL (±50.4), p=0.016 (Figure 4.3).

Repeated measures ANOVA showed no significant meal-stimulated changes in circulating GDF15 levels (p=0.08).

**Figure 4.1: Meal-stimulated plasma GDF15 levels in healthy volunteers**

![Diagram](image)

Figure 4.1 illustrating GDF15 levels over time A: Absolute values (pg/mL). The mean AUC for GDF15 from 0-180 minutes was 60670pg/mL (±5268, 95% CI 50344 to 70995). B: Change from baseline (delta values (pg/mL). Repeated measures ANOVA showed no significant meal-stimulated changes in circulating GDF15 levels (p=0.08). GDF15: Growth Differentiation Factor 15, AUC: Area under the curve.

### 4.2.3.3 Salivary GDF15

The mean fasted salivary GDF15 value was 63.8pg/mL (±80.3). The mean AUC for salivary GDF15 from 0-180 minutes was calculated at 10366pg/mL (±2820, CI 4839 to 15892). The trend in salivary GDF15 over time is illustrated in Figure 4.2.

At the end of tasting the meal, salivary GDF15 levels had dropped to 26.2pg/mL (±15.7), reflecting a mean reduction of -29.1pg/mL (±74.5), p=0.009 (Figure 4.2).
Repeated measures ANOVA showed no significant meal-stimulated changes in salivary GDF15 levels ($p=0.07$). Figure 4.3 illustrates a comparison between delta GDF15 values in plasma and saliva respectively.

**Figure 4.2: Meal-stimulated salivary GDF15 levels in healthy participants**

Figure 4.2 illustrating GDF15 levels over time A: Absolute values (pg/mL) The mean AUC for salivary GDF15 from 0-180 minutes was calculated at 10366pg/mL ($\pm 2820$, CI 4839 to 15892). B: Change from baseline (delta values (pg/mL). At the end of tasting the meal (T15) salivary GDF15 levels dropped to 26.2pg/mL ($\pm 15.7$), with a mean reduction of -29.1pg/mL ($\pm 74.5$), $p=0.009$. Repeated measures ANOVA showed no significant meal-stimulated changes in salivary GDF15 levels ($p=0.07$). GDF15: Growth Differentiation Factor 15, AUC: Area under the curve.
Figure 4.3: Meal-stimulated salivary and plasma GDF15 changes from baseline

Figure 4.3 illustrating the change in GDF15 levels from baseline (T0) over time (delta GDF15, pg/mL) in meal-stimulated plasma and saliva. The mean 15-minute GDF15 level was 337.9pg/mL (±106.5). This reflected a mean reduction from baseline of -129.3pg/mL (±50.4), p=0.016. Red filled circles represent plasma levels and blue filled squares saliva levels. GDF15: Growth Differentiation Factor 15, AUC: Area under the curve.

Figure 4.4: Change in plasma and salivary GDF15 following pre- and post- taste exposure from the study meal

Figure 4.4 illustrating the change in plasma and salivary GDF15 levels (Absolute values: pg/mL) following pre- (T0) and post- (T15) taste exposure from the study meal. (* indicates p<0.05) GDF15: Growth Differentiation Factor 15.
4.2.3.4 Relationship of GDF15 to body composition

Pearson’s correlation analyses were performed in order to investigate the relationship between plasma GDF15 levels and body composition (Table 4.3). Circulating GDF15 levels showed no significant correlation to BMI or body composition in this cohort of healthy weight adults. Similarly, salivary GDF15 levels also did not correlate with BMI nor parameters of body composition measured by BIA (Table 4.4). There was also no significant correlation between fasting salivary and fasting plasma GDF15 levels in this (Pearson’s r 0.1634, CI -0.4530 to 0.6741, p=0.611)

Table 4.3: Correlation analysis between fasted plasma GDF15 levels and parameters of body composition

<table>
<thead>
<tr>
<th></th>
<th>Plasma GDF15 vs BMI</th>
<th>Plasma GDF15 vs Fat %</th>
<th>Plasma GDF15 vs Fat mass (kg)</th>
<th>Plasma GDF15 vs FFM (kg)</th>
<th>Plasma GDF15 vs Muscle mass (kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pearson’s r</td>
<td>-0.243</td>
<td>-0.353</td>
<td>-0.164</td>
<td>0.252</td>
<td>0.283</td>
</tr>
<tr>
<td>95% CI</td>
<td>-0.700 to 0.355</td>
<td>-0.757 to 0.246</td>
<td>-0.656 to 0.426</td>
<td>-0.376 to 0.722</td>
<td>-0.317 to 0.722</td>
</tr>
<tr>
<td>R squared</td>
<td>0.059</td>
<td>0.125</td>
<td>0.027</td>
<td>0.063</td>
<td>0.0802</td>
</tr>
<tr>
<td>p value</td>
<td>0.423</td>
<td>0.237</td>
<td>0.593</td>
<td>0.430</td>
<td>0.349</td>
</tr>
</tbody>
</table>


Table 4.4: Correlation analysis between fasted salivary GDF15 levels and parameters of body composition

<table>
<thead>
<tr>
<th></th>
<th>Salivary GDF15 vs BMI</th>
<th>Salivary GDF15 vs Fat %</th>
<th>Salivary GDF15 vs Fat mass (kg)</th>
<th>Salivary GDF15 vs FFM (kg)</th>
<th>Salivary GDF15 vs Muscle mass (kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pearson’s r</td>
<td>-0.403</td>
<td>-0.106</td>
<td>-0.224</td>
<td>-0.145</td>
<td>-0.094</td>
</tr>
<tr>
<td>95% CI</td>
<td>-0.793 to 0.223</td>
<td>-0.641 to 0.498</td>
<td>-0.707 to 0.401</td>
<td>-0.685 to 0.498</td>
<td>-0.634 to 0.507</td>
</tr>
<tr>
<td>R squared</td>
<td>0.162</td>
<td>0.012</td>
<td>0.051</td>
<td>0.021</td>
<td>0.009</td>
</tr>
<tr>
<td>p value</td>
<td>0.194</td>
<td>0.744</td>
<td>0.483</td>
<td>0.670</td>
<td>0.771</td>
</tr>
</tbody>
</table>

4.2.4 Discussion

Study 1 compared paired plasma and salivary GDF15 levels, in the fasted state, after tasting food and over a 180-minute period following a meal in adults with a healthy weight. When interpreting GDF15 results, it is important to bear in mind the circadian rhythm of circulating GDF15 levels. Data from investigating the diurnal variability of circulating GDF15 levels, suggest this GDF15 levels follow a circadian pattern with levels peaking overnight and lowest levels seen in the early afternoon[31]. Research meal-studies on human research participants are commonly conducted in the morning and dips in GDF15 recorded in the late morning, which often coincides with time points 60-120 minutes following a study meal, are therefore likely to represent changed in diurnal variation[26]. In this study, meal-stimulated plasma GDF15 levels did not significantly differ over the 180-minute period of the study and were not altered by eating. This finding is in line a small number of studies on healthy adult volunteers have concluded that circulating GDF15 levels are not altered by acute changes in nutrient availability[27,31,32]. A study comparing circulating GDF15 levels to insulin and GLP-1 in healthy humans following a glucose load and a mixed meal, demonstrated significant changes in GDF15 in response to a meal and no relationship between circulating glucose, GLP-1 or insulin levels and GDF15[27].

In this study, we also compared the relationship between GDF15 levels to BMI and parameters of body composition. GDF15 has previously been shown to correlate with BMI, both in healthy weight adults and in people with overweight or obesity, including in twins[31,33]. In this study however no significant correlation was observed between GDF15 and BMI or GDF15 and any other parameters of body composition. This is likely a reflection of the low BMI and low range of BMI observed in this study’s cohort of predominantly very lean participants. Ethnicity may have had a further impact on this study’s results, as ethnic variabilities in GDF15 remain largely unknown.

To date, to our knowledge no studies have previously measured salivary GDF15 levels in the context of a meal or in response to eating. Salivary GDF15 has been measured in previous studies in the context of prognosticating outcomes in cardiac or malignant disease[28,34]. Salivary samples were collected immediately before and immediately
after the meal. Comparison between these two samples showed a significant drop following the meal (63.8pg/mL ±80.3 vs. 26.2pg/mL ±15.7 p=0.009). No other significant changes were detected over the 180-minute period of the study. The drop in salivary GDF15 at 15-30 minutes was no longer significant after adjusting for multiple comparisons in the ANOVA model, however it was close to the significance level (p=0.07) and this may be a reflection of the small number of participants in this study.

Similarly, regarding the suppression in circulating GDF15 at 15 minutes following a meal however, the 15 minute interval between the collection of these two samples is too short for this to represent a change due to circadian variability. Again, although when comparing the 15 minute sample to the baseline sample the suppression was significant, this did not maintain statistical significance following adjustment for multiple comparison in the ANOVA model comparing the trend in circulating GDF15 from 0 to 180 minutes. It is also important to note that neither of the two studies that previously investigated meal-stimulated GDF15 levels in healthy weight adults collected samples at 15 minutes following a meal. The first sample in both of these studies was collected at 30 minutes[27,31]. The significance of this reduction at 15 minutes and the physiological driver for this hence remains unclear. In light of the existing data concluding that GDF15 is not altered in response to acute changes in nutrient availability, as well as following intravenous glucose loads, it is unlikely that this represents a gut-mediated response to ingested nutrients. Given the concomitant salivary GDF15 reduction and the suggested role of GDF15 in taste preference to fat it is plausible that this may be mediated through signalling the presence of fatty acids in the oral cavity. More studies will however be required to elucidate this further.

4.3 Study 2: Meal-stimulated GDF15 levels in people with severe obesity

4.3.1 Hypothesis
In view of the fact that circulating GDF15 levels are not significantly altered by a meal in healthy weight adults, the hypothesis for this study is that circulating GDF15 will not be altered following a meal in people with severe obesity.
4.3.2 Methods

4.3.2.1 Recruitment and setting
Participants were recruited from the University College London Hospitals (UCLH) Bariatric Centre for Weight Management and Metabolic Surgery. Participation in the study was voluntary and informed written consent was obtained. The study had National Health Service Research Ethics Committee approval (ID#09/H0715/65) and was undertaken in accordance with the Helsinki Declaration.

4.3.2.2 Participants
Adult male and female patients with severe obesity, defined as a BMI ≥35kg/m² who were due to undergo a primary SG were invited to participate. The study’s exclusion criteria were T2DM, HIV and hepatitis B, pregnancy and use of medications known to affect weight. Given the aim of this study was to investigate the trend of GDF15 in this cohort of participants, no prior power calculations were undertaken and results were treated as exploratory.

4.3.2.3 Study assessments
Study visits took place at the UCLH Clinical Research Facility. Participants attended in the morning of their study visits following a 12h overnight fast. An intravenous cannula was inserted into a forearm vein and they were asked to rest for 45 minutes. Following the acclimatisation period, a fasted blood sample was obtained. Participants subsequently consumed a 500kcal liquid meal over a 10-minute period (Resource Fibre 2, Vanilla Flavour, 250mL). Blood samples were obtained at 15, 30, 60, 90, 120, 150 and 180 minutes following the meal.

GDF15 measurements and statistical analyses were performed as described in Study 1.

4.3.3 Results

4.3.3.1 Participant demographics
A total of 8 participants without T2DM or other metabolic conditions were included in this study. Their demographics can be seen in Table 4.5.

Table 4.5: Demographics of participant with severe obesity

<table>
<thead>
<tr>
<th>Participants with severe obesity (n=8)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Sex</strong></td>
</tr>
<tr>
<td>88.9% Female (n=7)</td>
</tr>
<tr>
<td>11.1% Male (n=1)</td>
</tr>
<tr>
<td><strong>Age (years)</strong></td>
</tr>
<tr>
<td>32.0 years (±3.9)</td>
</tr>
<tr>
<td><strong>BMI (kg/m²)</strong></td>
</tr>
<tr>
<td>42.9 (±4.4)</td>
</tr>
<tr>
<td><strong>Ethnicity</strong></td>
</tr>
<tr>
<td>87.5% (n=7) White</td>
</tr>
<tr>
<td>12.5% (n=1) Black/African/Caribbean</td>
</tr>
</tbody>
</table>

4.3.3.2 Meal-stimulated plasma GDF15 levels

The mean fasted GDF15 plasma value in this study cohort was 454.0pg/mL (±189.9). The mean AUC for GDF15 from 0-180 minutes was 81733pg/mL (±8377, 95% CI 65315 to 98151) and the trend of the plasma levels measured over time can be seen in Figure 4.5A.

The AUC of the change in GDF15 levels from baseline (delta values) was 1617pg/mL (±2020, CI 0 to 5576) and a graphical representation of the change from the baseline value over time can be seen in Figure 4.5B. ANOVA showed no significant meal-stimulated changes in plasma GDF15 levels (p=0.300).
Figure 4.5: Meal-stimulated plasma GDF15 levels in people with severe obesity

Figure 4.5 illustrating plasma GDF15 levels in people with severe obesity over time A: Absolute values (pg/mL) B: Change from baseline (delta values (pg/mL). The mean AUC for GDF15 from 0-180 minutes was 81733pg/mL (±8377, 95% CI 65315 to 98151). B; Change in plasma GDF15 from baseline over time (delta values). Repeated measures ANOVA showed no significant meal-stimulated changes in plasma GDF15 levels (p=0.300). Red filled circles indicate mean plasma levels, error bars reflect standard error of the mean. GDF15: Growth Differentiation Factor.

4.3.3.3 Relationship of GDF15 to body composition

Pearson’s correlation analyses were performed in order to investigate the relationship between circulating GDF15 levels BMI and age (Table 4.6). Circulating GDF15 levels strongly correlated with BMI (r 0.999, p<0.001).

Table 4.6: Correlation of GDF15 levels with age and BMI

<table>
<thead>
<tr>
<th></th>
<th>Plasma GDF15 vs BMI</th>
<th>Plasma GDF15 vs Age</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pearson’s r</td>
<td>0.9999</td>
<td>-0.777</td>
</tr>
<tr>
<td>95% CI</td>
<td>0.998 to 1.000</td>
<td>-0.995 to 0.7271</td>
</tr>
<tr>
<td>R squared</td>
<td>0.999</td>
<td>0.603</td>
</tr>
<tr>
<td>p value</td>
<td>&lt;0.001</td>
<td>0.223</td>
</tr>
</tbody>
</table>

GDF15: Growth Differentiation Factor, BMI: Body Mass Index.

4.3.4 Discussion

In this study, meal stimulated GDF15 levels in people with severe obesity were measured. No significant changes were observed following the meal (p=0.300). This is in line with findings from other studies both in people with healthy weight and in people with obesity[27,31,32]. In this cohort of participants with a mean BMI of
42.9kg/m², a significant correlation between BMI and GDF15 levels was observed, in keeping with the existing literature[31,33]. Furthermore, data from research on human adipose tissue has demonstrated that GDF15 is both expressed and produced by adipocytes[35]. It is therefore likely that the correlation between GDF15 with BMI is a manifestation of the increasing adipose tissue mass. Given the pro-inflammatory nature of GDF15, obesity-associated inflammation likely contributes to the rise in GDF15 with BMI further[36].

Interestingly, no suppression in GDF15 levels was seen at 15 minutes following the meal, which in contrast was observed in study one. This difference might be related to the liquid meal type used in this study compared to the solid meal of study 1, however more studies will be required in order to elucidate this further. The suppression seen at 120 minutes, although not statistically significant, is in line with the findings from Tsai et al., regarding circadian rhythm related suppressions in GDF15[31].

4.4 Study 3: Orosensory detection and preference for dietary fat in people with severe obesity

4.4.1 Hypotheses

1. Taste sensitivity to dietary fat in people with severe obesity differs in the fasted and fed state.
2. Taste sensitivity to dietary fat is altered by bariatric surgery, driving a change in food preference away from high-fat foods.
3. Salivary GDF15 levels directly respond to tasting dietary fat in the absence of energy intake, in a manner independent to circulating GDF15 levels.

4.4.2 Methods

4.4.2.1 Participants and recruitment
Participants were recruited from the UCLH Bariatric Centre for Weight Management and Metabolic Surgery. Participation in the study was voluntary and informed written
consent was obtained. The study had National Health Service Research Ethics Committee approval (ID#09/H0715/65) and was undertaken in accordance with the Helsinki Declaration.

Adult male and female patients with severe obesity, defined as a BMI ≥35kg/m², who had not yet commenced any formal weight loss intervention and were proficient in written and spoken English were invited to participate. Additional exclusion criteria included pregnancy and lactation and any acute or chronic condition known to affect gustatory and olfactory function, such as T2DM, B12 or zinc deficiency, smoking and any acute or chronic conditions that may affect the sense of smell or taste.

A second group of participants who had undergone a primary RYGB or SG and were within 5 to 12 months from their date of surgery were also recruited in order to compare the effects of bariatric surgery on taste sensitivity to dietary fat. In order to eliminate the effects of early post-operative restrictions in diet and to allow new eating behaviour to be established, patients who were under 5 months from their date of surgery were excluded. Percentage weight loss (%WL) was calculated using the following formula: %WL = [(day of surgery body weight - study visit body weight)/day of surgery body weight]*100. This study was designed to provide pilot data, recruitment was hence not based on a power calculation and results are treated as exploratory.

4.4.2.2 Study assessments

Study visits took place at the UCLH Clinical Research Facility. Participants arrived in the morning following an overnight fast. Participants were advised to refrain from consuming alcohol and strenuous exercise in the 24 hours prior to their study visit. Upon arrival, an intravenous cannula was inserted into a forearm vein and participants were advised to rest for 45 minutes. Height was recorded using a wall-mounted stadiometer, weight using calibrated weighing scales and BMI was calculated.

Following the acclimatisation period, a paired fasted plasma and saliva sample were collected (Time point F - fasted). Participants subsequently underwent a test to determine their taste sensitivity to fat, assessed via establishing their taste detection
threshold for oleic acid (C18:1). Taste detection thresholds for oleic acid was assessed using a validated forced choice methodology by Haryono et al.[37]. At the end of the test, a further paired blood and saliva sample were collected (Time point 0).

Participants then consumed a liquid 478kcal meal, divided into four portions with different fat contents and were asked to rank their respective pleasantness on a labelled magnitude scale (LMS) (Table 4.7) (LMS in appendix 1). Plasma and saliva samples were collected at 15, 30, 60, 90, 120, 150 and 180 minutes from the time of the meal. The taste test for sensitivity to fat was repeated 60 minutes following the meal in order to enable comparison between taste sensitivity between the fasted and fed state.

Table 4.7: Study meal and fat content

<table>
<thead>
<tr>
<th>Portion</th>
<th>Optifast</th>
<th>Calogen</th>
<th>Fat content</th>
<th>% fat</th>
</tr>
</thead>
<tbody>
<tr>
<td>Portion 1</td>
<td>50mL</td>
<td>-</td>
<td>1.125g</td>
<td>2.2%</td>
</tr>
<tr>
<td>Portion 2</td>
<td>50mL</td>
<td>10 mL (5g fat)</td>
<td>6.125g</td>
<td>12.25%</td>
</tr>
<tr>
<td>Portion 3</td>
<td>50mL</td>
<td>20mL (10g fat)</td>
<td>11.125g</td>
<td>22.25%</td>
</tr>
<tr>
<td>Portion 4</td>
<td>50mL</td>
<td>30mL (15g fat)</td>
<td>16.125g</td>
<td>32.25%</td>
</tr>
</tbody>
</table>

Table 4.7: Composition of the four meal portions. Optifast Vanilla flavoured milk shake was used as the base of the meal and Calogen (neutral flavour) was added to create portions of different fat concentrations. The meal volume was 260mls and had 478kcal.

4.4.2.3 Taste sensitivity for oleic acid

The samples used to assess oral oleic acid taste sensitivity were prepared using a previously validated method [37]. Oleic acid (Sigma-Aldrich) was diluted to varying concentrations (0.02, 0.06, 1, 1.4, 2, 2.8, 3.8, 5, 6.4, 8, 9.8, 12 and 20mM) with long-life fat-free milk. In order to eliminate textural cues from the addition of fatty acids and to create texturally similar samples, 5% w/v food grade gum acacia (Baldwin & co) and liquid paraffin (Care+) were added to all samples. In order to prevent oxidation of C18:1 0.01% w/v EDTA was added (Merck). Samples were homogenized and prepared fresh on the morning of the study and were given to participants at room temperature.
In order to prevent confounding olfactory inputs, taste tests were conducted wearing nose clips. Taste detection thresholds for oleic acid were determined using the ascending forced choice triangle methodology (Figure 4.6)[37]. The participant was presented with a set of three samples, one containing oleic acid and two controls. They were instructed to taste all three, without swallowing any of the sample. They were instructed to rinse their mouth using water. They were then asked to identify the sample that tasted different to the other two. If the oleic acid sample was correctly identified, the presented sample concentration remained the same in the next set of samples. If an incorrect sample was identified, the oleic acid sample concentration in the next three samples was increased to the next higher concentration. This process was repeated until the participant correctly identified the oleic acid sample at the same concentration three consecutive times. That concentration was defined as the participant’s oleic acid taste detection threshold.

**Figure 4.6: Ascending forced choice triangle methodology**

![Image](image_url)

Figure 4.6 illustrating the methodology used to determine taste detection thresholds for oleic acid using the ascending forced choice triangle[37]. mM: Millimolar, C18:1: Oleic Acid
4.4.2.4 Sample collection and processing
Samples were collected and processed following the procedures detailed in Study 1.

4.4.2.5 Statistical analysis
Statistical analysis was carried out as outlined in Study 1.

4.4.3 Results

4.4.3.1 Study population
A total of 10 people with severe obesity were recruited into the study, 9 participants were female and 1 was male. The mean age in the group was 44.3 (± 3.9) years and mean BMI was 45.5 kg/m² (±1.4). With regard to ethnicity, 55.6% (n=5) of participants were of White background and 44.4% (n=4) of Black/African/Caribbean background.

A second group of 10 participants who had bariatric surgery were recruited, 7 following a SG and 3 post-RYGB; their mean age was 40.5 (±4.6) years. With regard to ethnicity, 70% (n=7) of participants were of White background and 30% (n=3) of Black/African/Caribbean background. Four participants were male and 6 were female. Their mean day of surgery BMI was 47.4 (±2.6) and mean %WL from surgery 25.4% (±2.1), which is comparable to the mean %WL of patients undergoing bariatric surgery at UCLH[38]. The mean study day BMI was 35.6 (±2.5) and participants were a mean 8.4 months (±0.8) from their day of surgery. The BMI in the pre-surgery group was comparable to the pre-surgery BMI of the bariatric surgery group (47.9 vs. 45.5, p=0.441). However, as expected at the time of the study the post-surgery group had a significantly lower BMI than the pre-surgery group (p=0.008).

4.4.3.2 GDF15 plasma levels
Mean GDF15 levels for participants with severe obesity in this study were 617.7 pg/mL (±55.8) in the fasted state. Following the first taste test mean GDF15 levels were 631.1 pg/mL (±57.4), with no significant change in circulating GDF15 levels seen following the taste test. The total AUC for plasma GDF15 levels over the course of the study was 5047 (±405) with one positive peak demonstrated at 60 minutes following
the meal. However, ANOVA did not reveal any significant changes in GDF15 levels over time (P=0.347). At 60 minutes following the meal, and prior to the second taste test, mean plasma GDF15 levels were 654.2pg/mL (±65.9). At 90 minutes and following the second taste test results were 635.3pg/mL (±73.7), again showing no change in plasma levels following the second taste test. Plasma GDF15 levels over time are illustrated in Figure 4.7A.

4.4.3.3 Salivary GDF15 levels

Mean salivary GDF15 levels were 143.52pg/mL (±51.3) in the fasted state. Following the first taste test, at T0, salivary GDF15 suppressed to 13.4 pg/mL (±2.8) (p=0.016). Following the meal, levels rose to 79.0 pg/mL (±33.9) at 60 minutes following a meal. The second taste test, carried out after the T60 samples were obtained, also resulted in a suppression of GDF15 levels to 19.7pg/mL (±4.9). Salivary GDF15 levels returned to levels similar to baseline by 180 minutes, with a meal level of 149.9pg/mL (±71.1). The calculated AUC for salivary GDF15 levels was 483.7pg/mL (±168.5).

ANOVA revealed significant change in salivary GDF15 levels over time (p=0.016). Multiple comparisons showed a significant reduction from baseline (TF) at T0 (following the first taste test). No significant differences were observed over the 180-minute period at any of the other time points. Salivary GDF15 levels over time are illustrated in Figure 4.7B.
Figure 4.7: Plasma and salivary GDF15 levels over time in participants with severe obesity

Figure 4.7 illustrating GDF15 levels (pg/mL) over time (TF-180 minutes). Results represent mean GDF15 value in pg/mL. Taste tests for detection threshold to oleic acid carried out fasted (TF) and 60 minutes following a meal (T60). Meal consumed at T0. A. Plasma GDF15 levels (TF-T180) B. Salivary GDF15 levels (TF-180 minutes). C. Change in GDF15 levels from baseline in plasma (red) and saliva (blue) (TF-180 minutes). Red filled circles represent plasma levels and blue filled squares saliva levels. TF: Fasted Time Point, GDF15: Growth Differentiation Factor 15.

4.4.3.4 Oleic acid taste detection threshold in people with severe obesity

Mean fasted taste detection threshold for oleic acid was 3.9mM (±0.8) in participants with severe obesity. Post-meal, this increased to 8.9mM (±1.8) (Figure 4.8). 100% of participants were less sensitive in detecting oleic acid post-meal compared to their fasting detection threshold (p=0.003).
4.4.3.5 Oleic acid detection thresholds in people with severe obesity compared to people who have had bariatric surgery

In the bariatric surgery group, the mean detection threshold for oleic acid was 4.7mM (±1.2) fasted and 6.1mM (±1.9) post-meal (p=0.541). There was no difference between the mean fasted taste detection threshold (4.3mM vs. 5.1mM, p=0.617) or between the mean post-meal taste detection thresholds (9.1mM vs. 6.6mM, p=0.557) between the two groups. In the bariatric surgery group, in 3 (30%) participants the taste detection threshold for oleic acid was lower following a 478kcal meal, in 4 (40%) it remained unchanged and in 3 (30%) it was higher compared to their fasted detection threshold. Although the number of participants is too small to enable regression analysis, on reviewing this data there is no obvious relationship between procedure type, time from surgery or %WL to the taste detection threshold pre- and post-meal.

**Figure 4.8: Fasted and post-meal oleic acid taste detection thresholds**

![Graph showing taste detection thresholds for oleic acid in people with severe obesity (Obesity group) and following bariatric surgery (BS group) fasted and 60 minutes following a meal. 100% of participants (Obesity group) were less sensitive in detecting oleic acid post-meal compared to their fasting detection threshold (p=0.003). Data are mean and SD. (* indicated p<0.05). SD: Standard Deviation.]
4.4.3.6 Preference for dietary fat across different meal portions

Participants with severe obesity rated the meal portions with different fat contents with similar scores. In participants from the bariatric surgery group, the hedonic value reduced as fat content increased, although the differences in the ratings were not statistically significant (Table 4.8 and Figure 4.9).

Table 4.8: Hedonic value assigned to different fat contents in meal

<table>
<thead>
<tr>
<th>Fat content (%)</th>
<th>Severe obesity group</th>
<th>Bariatric surgery group</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.25</td>
<td>6.8% (±10.3)</td>
<td>12.0% (±9.0)</td>
</tr>
<tr>
<td>12.25</td>
<td>4.5% (±12.6)</td>
<td>6.3% (±12.9)</td>
</tr>
<tr>
<td>22.25</td>
<td>-3.0% (±14.8)</td>
<td>-1.5% (±14.3)</td>
</tr>
<tr>
<td>32.25</td>
<td>5.7% (±13.9)</td>
<td>-5.9% (±13.1)</td>
</tr>
<tr>
<td>ANOVA</td>
<td>p=0.747</td>
<td>p=0.810</td>
</tr>
</tbody>
</table>

Hedonic value assigned to each meal portion, each containing 2.25, 12.25, 22.25 and 32.25% fat respectively, as marked on the labeled magnitude scale, for pre-surgery and post-surgery groups.

Figure 4.9: Hedonic value assigned to meal portions of different fat contents

Figure 4.9 illustrating the mean hedonic value assigned to each of the meal portions with increasing fat content by each of the groups. The filled blue circles represent mean hedonic value assigned to each meal portion by people with severe obesity (Obesity group) and the red pen squares the group following bariatric surgery (BS group).
A Spearman correlation coefficient was calculated as 0.357 (p=0.500) for the group of participants with severe obesity, indicating a weak positive correlation between fat content and hedonic value. However, in the group of participants who had bariatric surgery, the Spearman correlation coefficient was -1 (p=0.01), indicating that bariatric surgery changes the hedonic value of dietary fat, showing a strong negative correlation between increasing dietary fat content and the hedonic value of the meal (Table 4.9).

Table 4.9: Ranking of meal fat content based on assigned hedonic value

<table>
<thead>
<tr>
<th>Fat Content</th>
<th>Severe obesity group</th>
<th>Bariatric surgery group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2.25%</td>
<td>12.25%</td>
</tr>
<tr>
<td>Rank</td>
<td>1</td>
<td>4</td>
</tr>
<tr>
<td>Spearman r</td>
<td>0.375</td>
<td>-1</td>
</tr>
</tbody>
</table>

Each individual’s responses on the labeled magnitude scale were converted to an individual ranking of the four different fat concentrations, from 1 (least liked) to 4 (most liked). Correlation analysis was performed and the spearman’s correlation co-efficient calculated.

4.4.4 Discussion

This study investigated taste sensitivity to dietary fat, perceived hedonic value of a meal with different fat contents, circulating and salivary GDF15 levels in people with severe obesity. It also compared taste sensitivity to dietary fat and preference for the different fat contents in the study meal between people with severe obesity and people who had bariatric surgery in the preceding 5 to 12 months.

In line with findings from Study 2, plasma circulating GDF15 levels did not change over the 180-minute study time period. There were no changes observed to plasma GDF15 levels, in response to tasting fat during the two taste tests, nor to the meal. These findings are in keeping with both the results of Study 2, but also the literature suggesting that circulating GDF15 levels do not respond to acute changes in energy availability, either in people of healthy weight or in people with obesity[27,31,32].

In contrast, salivary GDF15 levels dropped significantly following the first taste test, suggesting that exposure to dietary fat in the oral cavity, in the absence of an energy
intake leads to a reduction in GDF15 levels. When the taste test was repeated in the fed state however, the reduction was lower, and was no longer statistically significant. Taken together these findings suggest that, in people with obesity, the fed state alters the signalling processes responding to the presence of dietary fat in the oral cavity. This is combined with a reduction in taste sensitivity to oleic acid in the fed compared to the fasted state. As illustrated in Figure 4.7C, GDF15 levels in saliva at 60 minutes, immediately before the second taste test, were lower by a mean 64.4pg/mL (143.5 to 79.1pg/mL) compared to the fasted sample (time point F) prior to the original taste test. Data from animal studies suggest that GDF15 has an active role in driving food preference away from dietary fat[39,40]. It is therefore plausible that these differences between the fasted and fed state may be, at least partially, related to lower levels of salivary GDF15.

The results from the circulating and salivary GDF15 levels from this study (Figure 4.7C) also suggest that GDF15 production in saliva is independent from GDF15 secretion into the systemic circulation. During the taste test, participants tasted a number of samples containing oleic acid until their detection threshold was determined, thereby exposing their taste buds to repeated contact with fatty acids, in the absence of any energy intake. This suggests that the presence of GDF15 in the oral cavity is able to suppress GDF15 in saliva. The origin of GDF15 in saliva and the exact mechanisms of its secretion remain unclear. GDF15 is known to be expressed both in salivary glands and in the tongue[41-43]. Although it is not known whether GDF15 is expressed by taste receptors, the fact that taste buds able to detect the presence of fatty acids express GLP-1, ghrelin and CCK suggests that these cells may have an innate ability to regulate appetite[44,45]. Although it is not yet known whether taste bud cells are able to directly affect GDF15 secretion, it seems biologically plausible that taste stimulation by fatty acids can generate a cephalic response, suppressing GDF15 in saliva. Interestingly, at a cellular level, oleic acid has been shown to directly stimulate GLP-1 production, a gut hormone which has an attenuated secretion profile in people with obesity[10]. Therefore, it is plausible that in a similar way, despite persistently elevated plasma GDF15 levels, the responsiveness of salivary GDF15 in people with obesity may be reduced.
The findings of the oleic acid taste testing in this study suggest that, in people with obesity, taste sensitivity to fat declines with ongoing exposure to dietary fat throughout a meal. Together with this, we also observed no differences in the preference of the different meal portions used in this meal in people with obesity. Interestingly, this reduction in taste sensitivity to fat in the fed state is no longer seen in people who have had bariatric surgery. In contrast, the group of participants who had bariatric surgery, consistently ranked the meal portion with the highest fat content as the least palatable and the Spearman rank correlation analysis demonstrated a strong negative correlation between increasing fat content in the meal and the assigned hedonic value. Both a preference for foods with a high dietary fat content in people with severe obesity and a food preference away from high fat foods in people who have had bariatric surgery have previously been shown[6,46,47]. The underlying physiological drivers for this change however are not yet understood. To date the effect of bariatric surgery on salivary GDF15 levels has not yet been shown. However, modulating preference for dietary fat or increasing the taste sensitivity by altering GDF15 levels pose attractive therapeutic strategies for people with obesity.

The main limitations of this study are its cross-sectional nature and the small numbers of participants. Results must therefore be treated as exploratory. The findings of this study however suggest that GDF15 in saliva may have a role in signalling the presence of dietary fat that is detected in the oral cavity. Further studies are therefore warranted, in order to understand the signalling mechanisms generated in response to tasting fatty acids, as well as the exact role of GDF15 in both plasma and saliva, both in obesity and following bariatric surgery.
4.5 Study 4: Circulating GDF15 levels in people with severe obesity before and after RYGB or SG and correlation with food preference

4.5.1 Hypotheses

1. RYGB and SG alter GDF15 secretion.
2. The post-bariatric surgery GDF15 secretion profile differs in people with and without T2DM.
3. The change in GDF15 secretion is related to post-operative changes in taste and food aversions.

4.5.2 Methods

4.5.2.1 Recruitment and setting
Participants were recruited from the UCLH Bariatric Centre for Weight Management and Metabolic Surgery. Participation in the study was voluntary and informed written consent was obtained. The study had National Health Service Research Ethics Committee approval (ID#09/H0715/65) and was undertaken in accordance with the Helsinki Declaration.

4.5.2.2 Participants
Adult male and female patients with severe obesity, defined as a BMI ≥35kg/m² who were due to undergo a primary SG or RYGB were invited to participate. The study’s exclusion criteria were HIV and hepatitis B, pregnancy and lactation and any acute or chronic conditions likely to impact upon post-operative weight loss. Participants with T2DM with a disease duration greater than 10 years and those treated with insulin were excluded. Eligible recruited participants were included in the study. Given the degree of change in GDF15 anticipated to be observed following bariatric surgery was not known, recruitment was not based on a formal power calculation and the results were treated as exploratory.

4.5.2.3 Study assessments
Study visits took place at the UCLH Clinical Research Facility. Participants attended for their baseline visit within two weeks from their data of surgery and for their follow up visits at 6 weeks, 6 months, and 12 months post-surgery. A fasted blood sample was obtained from participants at each time point. Weight was recorded using a validated weighing scale, height was measured using a wall-mounted stadiometer and BMI was calculated. At post-operative study visits, %WL was calculated.

4.5.2.4 Taste questionnaire
At each of the post-surgery follow-up visits, participants completed a questionnaire developed by Tichansky et al., and modified by Graham et al. enquiring about changes in their sense of smell, their sense of taste, any changes in food preferences and the presence of food aversion [48,49](Appendix).

4.5.2.5 Sample collection and processing
Sample collection and processing were undertaken as outlined for Study 1.

4.5.2.6 Statistical analysis
Statistical analysis was carried out as per Study 1. In addition, Pearson’s correlation analyses were carried out in order to investigate potential correlation between GDF15 and BMI, as well as post-operative weight loss. Linear regression analyses were also performed in order to assess the relationship between GDF15 and post-operative %WL.

4.5.3 Results

4.5.3.1 Participant demographics
A total of 66 participants completed this study. Of these, 45 did not have T2DM at recruitment and out of those, 32 underwent a SG and 13 a RYGB. A total of 21 participants with T2DM participated in the study, 15 had a SG and 6 a RYGB. Their respective demographics can be seen in Table 4.10. Participants were comparable in terms of sex, age and baseline BMI.
Table 4.10: Participant demographics

<table>
<thead>
<tr>
<th></th>
<th>Participant without T2DM</th>
<th></th>
<th>Participates with T2DM</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SG (n=32)</td>
<td>RYGB (n=13)</td>
<td>SG with T2DM (n=15)</td>
</tr>
<tr>
<td><strong>Sex</strong></td>
<td>93.3% Female (n=28)</td>
<td>100% Female</td>
<td>80.0% Female (n=12)</td>
</tr>
<tr>
<td></td>
<td>6.7% Male (n=2)</td>
<td></td>
<td>20.0% Male (n=3)</td>
</tr>
<tr>
<td><strong>Age (years)</strong></td>
<td>43.9 (±10.5)</td>
<td>51.5 (±9.6)</td>
<td>49.6 (±12.6)</td>
</tr>
<tr>
<td><strong>BMI (kg/m²)</strong></td>
<td>44.9 (±6.2)</td>
<td>45.4 (±4.6)</td>
<td>45.5 (±6.1)</td>
</tr>
<tr>
<td><strong>Ethnicity</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>White</strong></td>
<td>71.9% (n=23)</td>
<td>53.8% (n=7)</td>
<td>60.0% (n=9)</td>
</tr>
<tr>
<td><strong>Black/African/Caribbean</strong></td>
<td>12.5% (n=4)</td>
<td>30.8% (n=4)</td>
<td>13.3% (n=2)</td>
</tr>
<tr>
<td><strong>Asian/Asian British/Asian Indian</strong></td>
<td>0%</td>
<td>7.7% (n=1)</td>
<td>6.7% (n=1)</td>
</tr>
<tr>
<td><strong>Other</strong></td>
<td>6.3% (n=2)</td>
<td>7.7% (n=1)</td>
<td>6.7% (n=1)</td>
</tr>
<tr>
<td><strong>Not disclosed</strong></td>
<td>9.3% (n=3)</td>
<td>0%</td>
<td>13.3% (n=2)</td>
</tr>
</tbody>
</table>

SG: Sleeve Gastrectomy, RYGB: Roux-en-Y Gastric Bypass, BMI: Body Mass Index. T2DM: Type 2 Diabetes Mellitus.

Table 4.11 summarises the %WL achieved by each group of participants at each of their follow-up study visits following bariatric surgery.
Table 4.11: Percentage weight loss at study visits

<table>
<thead>
<tr>
<th></th>
<th>Participant without T2DM</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SG (n=32)</td>
<td>RYGB (n=13)</td>
<td>p value</td>
<td></td>
</tr>
<tr>
<td>%WL Visit B, 6 weeks post-surgery (%)</td>
<td>11.6 (±3.5)</td>
<td>9.7 (±3.0)</td>
<td>0.026</td>
<td></td>
</tr>
<tr>
<td>%WL Visit C, 6 months post-surgery (%)</td>
<td>24.0 (±5.6)</td>
<td>21.3 (±4.4)</td>
<td>0.144</td>
<td></td>
</tr>
<tr>
<td>%WL Visit D, 12 months post-surgery (%)</td>
<td>27.4 (±7.9)</td>
<td>27.8 (±5.5)</td>
<td>0.895</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Participants with T2DM</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>SG with T2DM (n=15)</td>
<td>RYGB with T2DM (n=6)</td>
<td>p value</td>
<td></td>
</tr>
<tr>
<td>%WL Visit B, 6 weeks post-surgery (%)</td>
<td>11.4 (±3.6)</td>
<td>11.1 (±2.4)</td>
<td>0.910</td>
<td></td>
</tr>
<tr>
<td>%WL Visit C, 6 months post-surgery (%)</td>
<td>21.0 (±5.3)</td>
<td>24.2 (±4.75)</td>
<td>0.125</td>
<td></td>
</tr>
<tr>
<td>%WL Visit D, 12 months post-surgery (%)</td>
<td>24.2 (±6.4)</td>
<td>29.5 (±4.5)</td>
<td>0.080</td>
<td></td>
</tr>
</tbody>
</table>


4.5.3.2 GDF15 levels at baseline

Mean GDF15 levels at baseline were 601.5pg/mL (±262.4) in participants without T2DM and 1069pg/mL (±517.1) in participants with T2DM. Baseline GDF15 levels were significantly higher in people with T2DM (p<0.001) (Figure 4.10).

There were no significant difference in baseline GDF15 levels between participants without T2DM in the SG group compared to the RYGB group (623pg/mL ±281.7 vs. 584.3 ±226.1, p=0.686). Similarly, there were no significant differences between baseline GDF15 levels in people with T2DM who underwent a SG compared to those with T2DM who had a RYGB (977pg/mL ±500.0 vs. 1299 ±530.0, p=0.205). Post-surgery
GDF15 levels were analysed together for participants who underwent RYGB and SG without and with T2DM respectively.

**Figure 4.10: Comparison of baseline GDF15 levels in participants with and without T2DM**

![Baseline GDF15 levels](image)

Figure 4.10 illustrating baseline plasma GDF15 levels (Absolute values: pg/mL) in participants with and without T2DM. Mean GDF15 levels were 601.5pg/mL (±262.4) in participants without T2DM and 1069pg/mL (±517.1) in participants with T2DM (p<0.001). (* indicates p<0.05). GDF15: Growth Differentiation Factor 15, T2DM: Type 2 Diabetes Mellitus.

**4.5.3.3 Correlation between baseline GDF15 and BMI**

At baseline plasma GDF15 levels showed a significant correlation with BMI both in participants without and with T2DM (Table 4.12). GDF15 significantly correlated with age in people with T2DM but not in the group of participants without T2DM.
Table 4.12: Correlation of GDF15 levels with age and BMI

<table>
<thead>
<tr>
<th></th>
<th>Plasma GDF15 vs BMI</th>
<th>Plasma GDF15 vs Age</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Participants without T2DM</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pearson’s r</td>
<td>0.305</td>
<td>0.063</td>
</tr>
<tr>
<td>95% CI</td>
<td>0.005 to 0.555</td>
<td>-0.242 to 0.357</td>
</tr>
<tr>
<td>R squared</td>
<td>0.093</td>
<td>0.004</td>
</tr>
<tr>
<td>p value</td>
<td>0.046</td>
<td>0.688</td>
</tr>
<tr>
<td><strong>Participants with T2DM</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pearson’s r</td>
<td>0.543</td>
<td>0.657</td>
</tr>
<tr>
<td>95% CI</td>
<td>0.145 to 0.790</td>
<td>0.314 to 0.848</td>
</tr>
<tr>
<td>R squared</td>
<td>0.295</td>
<td>0.432</td>
</tr>
<tr>
<td>p value</td>
<td>0.011</td>
<td>0.001</td>
</tr>
</tbody>
</table>

GDF15: Growth Differentiation Factor 15, T2DM: Type 2 Diabetes Mellitus.

4.5.3.4 GDF15 levels following bariatric surgery

Repeated measures ANOVA showed a significant change in GDF15 levels in participants without T2DM following bariatric surgery (p<0.001). Mean GDF15 levels at 6 weeks from surgery increased from 601.5pg/mL ±262.4 to 707.1pg/mL ±269.4 (p=0.029). By 6 months from surgery levels reduced to 593.9 ±217.8 and from 6 to 12 months GDF15 levels reduced further to 553.3pg/mL ±206.8 (Figure 4.11, Table 4.13).

In participants with T2DM, repeated measures ANOVA showed significant changes in GDF15 over time following bariatric surgery (p=0.003). No significant change was seen in GDF15 levels from baseline to 6 weeks post-surgery (GDF15 1069pg/mL ±517.1 to 1028pg/mL ±562.1, p>0.999). At 6 months from surgery GDF15 levels reduced to 816.5pg/mL ±332.2 and at 12 months to 749.5pg/mL ±265.7, which reflects a significant reduction from baseline to 6 months (p=0.022) and from baseline to 12 months (p=0.008). At 12 months from surgery GDF15 levels remained higher in the group of participants with T2DM compared to those without T2DM (553.3pg/mL ±206.8 vs 749.5pg/mL ±265.7, p=0.002).
### Table 4.13: Bonferroni multiple comparisons of GDF15 levels over time

<table>
<thead>
<tr>
<th></th>
<th>Mean difference</th>
<th>95% CI of difference</th>
<th>Adjusted p value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Participants without T2DM</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline vs. 6 weeks post-surgery</td>
<td>-105.6</td>
<td>-204.2 to -6.9</td>
<td><strong>0.029</strong></td>
</tr>
<tr>
<td>Baseline vs. 6 months post-surgery</td>
<td>7.7</td>
<td>-69.4 to 84.8</td>
<td>&gt;0.9999</td>
</tr>
<tr>
<td>Baseline vs. 12 months post-surgery</td>
<td>48.2</td>
<td>-32.2 to 128.6</td>
<td>0.6287</td>
</tr>
<tr>
<td>6 weeks vs. 6 months post-surgery</td>
<td>113.2</td>
<td>21.2 to 205.3</td>
<td><strong>0.008</strong></td>
</tr>
<tr>
<td>6 weeks vs. 12 months post-surgery</td>
<td>153.8</td>
<td>65.0 to 242.5</td>
<td><strong>0.001</strong></td>
</tr>
<tr>
<td>6 months vs. 12 months post-surgery</td>
<td>40.53</td>
<td>-19.9 to 101.0</td>
<td>0.425</td>
</tr>
<tr>
<td><strong>Participants with T2DM</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline vs. 6 weeks post-surgery</td>
<td>41.38</td>
<td>-274.9 to 357.6</td>
<td>&gt;0.9999</td>
</tr>
<tr>
<td>Baseline vs. 6 months post-surgery</td>
<td>252.7</td>
<td>27.4 to 478.0</td>
<td><strong>0.022</strong></td>
</tr>
<tr>
<td>Baseline vs. 12 months post-surgery</td>
<td>319.7</td>
<td>68.6 to 570.7</td>
<td><strong>0.008</strong></td>
</tr>
<tr>
<td>6 weeks vs. 6 months post-surgery</td>
<td>211.3</td>
<td>-45.4 to 468.1</td>
<td>0.155</td>
</tr>
<tr>
<td>6 weeks vs. 12 months post-surgery</td>
<td>278.3</td>
<td>-12.8 to 569.4</td>
<td>0.067</td>
</tr>
<tr>
<td>6 months vs. 12 months post-surgery</td>
<td>66.97</td>
<td>-39.7 to 173.7</td>
<td>0.487</td>
</tr>
</tbody>
</table>

GDF15: Growth Differentiation Factor 15, T2DM: Type 2 Diabetes Mellitus.

Baseline HbA1c in the T2DM group prior to surgery was 6.7% ±0.9 and 66.7% of participants were on oral pharmacotherapy for T2DM. At 12 months from surgery mean HbA1c was 5.6% ±0.4 (p<0.001) and 1 participant (4.3%) were taking glucose-lowering medications. There was one further participant with partial T2DM remission (4.3%) and all other participants achieved full T2DM remission at 12 months. Due to the low number of participants with no remission, it was not possible to compare GDF15 levels in people with and without T2DM remission.
Figure 4.11: GDF15 levels before and after bariatric surgery in participants without (A) and with T2DM (B)

Figure 11 illustrating GDF15 levels before (Baseline) and 6 weeks, 6 months and 12 months post bariatric surgery in participants without (A) and with (B) T2DM. At 12 months from surgery GDF15 levels remained higher in the group of participants with T2DM compared to those without T2DM (553.3pg/mL ±206.8 vs 749.5pg/mL ±265.7, p=0.002). (* indicates p<0.05, ** p<0.01 and *** p<0.001). GDF15: Growth Differentiation Factor 15, T2DM: Type 2 Diabetes Mellitus.

Figure 4.12: Change from baseline (delta values) in participants without and with T2DM following bariatric surgery

Figure 12 illustrating changes in GDF15 levels (delta values (pg/mL) over time from baseline (pre-bariatric surgery) to 6 weeks, 6 months and 12 months post bariatric surgery in participants with T2DM (represented by filled green squares) and those without T2DM (represented by filled orange circles). Error bars: SEM. GDF15: Growth Differentiation Factor 15, T2DM: Type 2 Diabetes Mellitus, SEM: Standard error of the mean.
4.5.3.5 Correlation of GDF15 to age and BMI post-surgery

At 12 months from surgery, GDF15 no longer correlated with BMI in participants without T2DM (Pearson’s r -0.1295, R squared 0.017, CI -0.408 to 0.171, p=0.397) nor in participants with T2DM (Pearson’s r 0.424, R squared 0.179, CI. -0.010 to 0.723, p=0.060).

4.5.3.6 Variability of GDF15 response and relationship to post-operative weight loss

Despite the trends in mean GDF15 levels described above, a considerable variability of GDF15 response to bariatric surgery was observed. Therefore, GDF15 differences from baseline to 12 months from surgery were further evaluated. Out of 45 participants without T2DM, 57.8% (n=26) had a reduction in GDF15 levels compared to baseline (mean reduction -173.3pg/mL). This reflected a mean reduction of 24.1% from baseline. The other 42.2% (n=19) had a rise in GDF15 levels compared to their pre-baseline result (mean increase 25.2pg/mL), reflecting mean increase of 29.5% in GDF15. Comparison of mean GDF15 levels in people who had a decrease in GDF15 from baseline at 12 months, compared to those who had an increase in GDF15 levels, revealed that baseline GDF15 levels were significantly higher at baseline in people whose levels dropped following surgery, compared to participants whose GDF15 levels rose following surgery (698.0pg/mL ±292.0 vs. 469.5pg/mL ±134.5, p=0.003). Pearson’s correlation analyses revealed a significant correlation between the reduction in GDF from baseline to 12 months and %WL ($r^2$=0.329, CI -0.7862 to -0.2392, p=0.002). In contrast, in participants who had a rise in GDF15 from baseline to 12 months, the correlation between change in GDF15 and %WL was not significant ($r^2$=0.171, CI -0.05062 to 0.7303, p=0.08).

Out of the 21 participants with T2DM, 76.2% (n=16) had a decline in their GDF15 levels from baseline (mean reduction -445.0pg/mL ±367.3), reflecting a mean reduction of 32.2%. The remaining 23.8% of participants there was a mean increase of 15.6% from baseline (mean increase 81.3pg/mL ±50.8). The mean weight loss was not significantly different between the participant with an increase versus a decrease in GDF15 from baseline (26.3% ±6.3 vs. 25.3% vs 7.3, p=0.767). No significant correlation was seen
between reduction in GDF15 and %WL ($r^2=0.098$, CI -0.216 to 0.700, $p=0.238$) or between increase in GDF15 and %WL ($r^2=0.017$, CI -0.908 to 0.849, $p=0.832$) at 12 months from surgery.

The relationship between baseline GDF15 and %WL was examined. In participants without T2DM, no significant correlation was observed between baseline GDF15 and %WL at 12 months ($r^2=0.077$, $p=0.060$). Furthermore, in participants without T2DM, the rise in GDF15 at 6 weeks showed a significant correlation to %WL at 12 months from surgery ($r^2=0.118$, $p=0.020$).

Linear regression analyses were performed in order to investigate the relationship between GDF15 following bariatric surgery and %WL at 12 months. In participants without T2DM, the reduction in GDF15 from baseline to 12 months from surgery predicted post-operative %WL at 12 months, in a model adjusting for age, sex, baseline BMI and procedure type (Table 4.14). The same effect was not observed in people with T2DM (Table 4.15).

Table 4.14: Linear regression analysis for %WL at 12 months from surgery in participants without T2DM

<table>
<thead>
<tr>
<th>Variable</th>
<th>B</th>
<th>95% CI (lower)</th>
<th>95% CI (upper)</th>
<th>B standardized</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex</td>
<td>-6.963</td>
<td>-16.371</td>
<td>2.446</td>
<td>-0.237</td>
<td>0.142</td>
</tr>
<tr>
<td>Age</td>
<td>0.120</td>
<td>-0.104</td>
<td>0.344</td>
<td>0.174</td>
<td>0.285</td>
</tr>
<tr>
<td>Baseline BMI</td>
<td>0.220</td>
<td>-0.239</td>
<td>0.669</td>
<td>0.989</td>
<td>0.329</td>
</tr>
<tr>
<td>Surgical procedure</td>
<td>-0.585</td>
<td>-0.338</td>
<td>-5.995</td>
<td>-0.053</td>
<td>4.279</td>
</tr>
<tr>
<td><strong>Delta GDF15</strong> (Baseline to 12 months)</td>
<td>-0.015</td>
<td>-0.028</td>
<td>-0.002</td>
<td>-0.394</td>
<td><strong>0.025</strong></td>
</tr>
<tr>
<td><strong>Delta GDF15</strong> (6 weeks to 12 months)</td>
<td>0.007</td>
<td>-0.005</td>
<td>0.018</td>
<td>0.199</td>
<td>0.024</td>
</tr>
</tbody>
</table>

GDF15: Growth Differentiation Factor 15, CI: Confidence Interval
4.5.3.7 Relationship between GDF15, taste changes and food aversions following bariatric surgery

The frequency of reported changes in taste and food aversions can be seen in Table 4.16.

Table 4.16: Reported changes in the sense of taste and food aversions

<table>
<thead>
<tr>
<th></th>
<th>6 weeks</th>
<th>6 months</th>
<th>12 months</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Participants without T2DM (n=45)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Changes in taste</td>
<td>82.2% (n=37)</td>
<td>75.6% (n=34)</td>
<td>73.3% (n=33)</td>
</tr>
<tr>
<td>Food aversions</td>
<td>51.1% (n=23)</td>
<td>71.1% (n=32)</td>
<td>73.3% (n=33)</td>
</tr>
<tr>
<td><strong>Participants with T2DM (n=21)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Changes in taste</td>
<td>61.9% (n=13)</td>
<td>81.0% (n=17)</td>
<td>71.4% (n=15)</td>
</tr>
<tr>
<td>Food aversions</td>
<td>57.1% (n=12)</td>
<td>57.1% (n=12)</td>
<td>57.1% (n=12)</td>
</tr>
</tbody>
</table>

T2DM: Type 2 Diabetes Mellitus

At 6 weeks from surgery, no significant differences were detected in the change from baseline in GDF15 levels in participants without T2DM with and without changes in
their sense of taste (Change in GDF15 143.5pg/mL ±280.4 vs 97.39 ±233.2, p=0.993), or food aversions (Change in GDF15 128.4pg/mL ±271.2 vs 79.39 ±230.9, p=0.984).

At 6 months from surgery, mean GDF15 change from baseline in participants with changes in their sense of taste was -24.1pg/mL ±186.9 compared to +43.1pg/mL ±187.5 in participants with no taste changes (p=0.306). In participants with food aversion the change in GDF15 from baseline was -4.2pg/mL ±194.3 compared to 25.5 ±176.3 in participants with no food aversions (p=0.669). Similarly, no relationship between change in GDF15 from baseline and taste changes at 12 months were seen in participants without T2DM (-26.6 ±185.4 vs. -56.1 ±200.9, p=0.660) or food aversions at 12 months (-3.9pg/mL ±148.0 vs -66.7 ±209.9, p=0.366).

The above analysis was repeated for participants with T2DM and no significant changes were seen between the change in GDF15 following bariatric surgery and the presence of taste changes or food aversions.

4.5.4 Discussion
This study compares GDF15 levels in 66 people with severe obesity, with and without T2DM, longitudinally prior to and at 6 weeks, 6 months and 12 months following bariatric surgery. Participants with T2DM had significantly higher GDF15 levels compared to euglycaemic participants (601.5pg/mL ±262.4 vs. 1069.0pg/mL ±517.1, p<0.001). This difference is in line with the observation that people with obesity and T2DM have higher GDF15 levels compared to people of the same BMI without T2DM[50,51]. Increased VAT mass, insulin resistance and endothelial dysfunction, which directly stimulates GDF15 release, have all been proposed as mechanisms that lead to increased GDF15 levels in people with T2DM and obesity, beyond the association with BMI[52,53]. In both of this study’s cohorts, GDF15 levels significantly correlated with BMI and in the T2DM group also with age. The absence of a significant correlation with age in the participant group without T2DM is likely to reflect a narrower age range in this group.
Following bariatric surgery, significant changes in GDF15 levels were observed, both in people with and without T2DM. In participants without T2DM, a significant rise was seen from baseline to 6 weeks after surgery. Subsequently, levels dropped, with significant reductions observed from 6 weeks to 6 months and from 6 weeks to 12 months. In participants with T2DM, no significant changes were seen from baseline to 6 weeks from surgery. Significant reductions in GDF15 levels were however seen from baseline to 6 months and from baseline to 12 months. The transient elevation in GDF15 seen in our group without T2MD is likely to be due to the post-surgical catabolic state, lipolysis and rapid weight loss seen in early months following surgery and has also been reported in the two other studies that have investigated GDF15 secretion following bariatric surgery[25,54]. Both these studies, however, reported elevations in GDF15 following RYGB and SG respectively beyond the original post-operative follow up period. Kleinert et al., who investigated the effects of RYGB on a group of 25 people without and 22 with T2DM, at 1 week, 3 months, 1 year and 2.5-4 years from RYGB, concluded that RYGB was associated with increases in GDF15 which correlated with weight loss[25]. This study’s results however do not represent findings from a longitudinally followed up cohort of patients and each time point, but instead they originate from samples collected from three different study cohorts. Furthermore, the authors have analysed results from participants with and without T2DM together, which may allow for the impact of T2DM to skew the interpretation of their results. Dolo et al., investigated GDF15 levels following SG in 21 people with obesity (15 without and 6 with T2DM)[1]. In this study, where trends were again reported together for people with and without T2DM, an elevation of GDF15 levels at 1 month and 3 months post-SG, with levels that remained elevated at 12 months. The authors conclude that elevations in GDF15 may be driving post-surgery appetite suppression in people following SG. Of note, in this study is the very low GDF15 levels seen prior to surgery in this cohort, with a mean GDF15 level of 215.5pg/mL, despite a mean BMI of 43kg/m²[13]. In comparison, baseline GDF15 levels in our study at baseline were 601.5pg/mL in people without and 1069pg/mL in participants with T2DM. Similarly, in our study 2 discussed above, which was performed on a different cohort of patients without T2DM, baseline GDF15 levels were 617.7pg/mL. The study by Dolo et al. was conducted in China and ethnicity-related difference may explain the lower baseline
GDF15 levels despite the similar BMI to our cohorts. A meta-analysis investigating the predictive value of GDF15 in acute coronary syndrome, has previously highlighted the lower GDF15 levels seen in Asian cohorts[55]. The impact of these ethnic differences and how this relates to the physiological impact of GDF15 on appetite, body weight, food preference, as well as the responses to bariatric surgery remain unknown. The other study that investigated a change in GDF15 secretion following SG was conducted by Salman et al. and showed that SG lead to a significant increase in GDF15 at 12 months[56]. This study again included people with and without T2DM and showed a significant correlation between the increase in GDF15 and the reduction in kg of weight. %WL was however not reported by the authors. It also has to be noted that 27.9% of the participants in that study had T2DM at baseline and only 14.9% had achieved T2DM remission at 12 months, a factor which could have a considerable impact on persistently elevated GDF15 levels.

This study, to our knowledge, is the first to separately investigate GDF15 levels before and after bariatric surgery in people with and without T2DM. Baseline GDF15 levels before bariatric surgery were significantly higher in people with T2DM, which is consistent with reports in the literature[37]. No significant rise was observed at 6 weeks and significant reductions in GDF15 were seen at 6 and 12 months, which may be a reflection of the improved glycaemic status, given all participants in this cohort achieved remission of T2DM following bariatric surgery. In addition, withdrawal of metformin, which is known to increase expression of GDF15, may have a further impact upon changes in GDF15 levels following bariatric surgery[57]. In order to investigate the effect of bariatric surgery without the confounding influence of treatment withdrawal, studies would need to be undertaken on people newly diagnosed with T2DM, undergoing surgery prior to initiating treatment with oral antihyperglycemics, which was not feasible in this cohort.

The response in GDF15 secretion to bariatric surgery in this study was not universal and considerable variability exists between individuals. Interestingly, although an overall reduction in GDF15 was observed in the group of participants without T2DM following bariatric surgery, this was only the case for 57.8% of participants. In the
remaining 42.2% GD15 levels rose following bariatric surgery. However, GDF15 levels at baseline were higher at baseline in people who had a reduction in GDF15 at 12 months from surgery, compared to those who had an increase in GDF15 at 12 months. This may reflect differences in both underlying physiology, but also in the response to secretion profiles following bariatric surgery. For instance, despite its role in appetite and food preference, GDF15 is a pro-inflammatory cytokine and promotes metabolic adaptation to inflammation, hence higher baseline levels in a subset of participants with obesity may reflect the presence of a pro-inflammatory state[58]. In addition, GDF15 is responsive to circulating levels of triglycerides[58]. The reduction in GDF15 seen in this study may therefore reflect a weight loss related improvement in an obesity-associated inflammatory state. Potential links between GDF15 and resolution of obesity-related chronic inflammation are further discussed in Chapter 6. It is, however, important to note that, in this study, the reduction in GDF15 from baseline to 12 months, correlated with and predicted post-operative 12 month %WL.

Variability in GDF15 responses to obesity interventions have previously been reported. A study investigating the impact of an exercise intervention in adults with obesity, highlighted that the same intervention had different impact on GDF15 levels between participants in the study[59]. The authors showed a reduced preference for high-fat foods in participants who had an exercise-induced rise in GDF15 compared to those who had a reduction in GDF15[59,60]. Furthermore, Cai et al. investigated the effect of a 3-week lifestyle intervention on GDF15 levels in young adults with obesity and showed that this led to an increase in GDF15 in 77% and a reduction in 23%[61]. Similarly, although both studies reported an overall increase in GDF15, both the studies by Dolo at al. and Salman et al. included a large variability of GDF15 following bariatric surgery, although this was not commented on by the authors[54,56].

In this study, no relationship between GDF15 levels and taste changes or food aversion following bariatric surgery was seen. These results may have been partially affected by the very low numbers of participants in this study who did not experience food aversions or taste changes in this study. It is however important to note that irrespective of the impact of GDF15 on appetite following bariatric surgery, its role in
appetite physiology is not homeostatic[62]. Furthermore, animal studies have demonstrated that despite its beneficial effects on appetite, GDF15 is not required for weight loss following SG in mice[20]. A substantive body of evidence suggests that bariatric surgery alters signals of energy homeostasis, notably gut hormones including GLP-1 and PYY, which in addition to having a role in energy homeostasis also control taste physiology and over-secretion has been linked to food aversions[63-65]. Studies investigating the relationship between the action of GDF15 and gut hormones suggest that these exhibit their anorectic effects through different pathways. Although GDF15 has been demonstrated to act synergistically with GLP-1 analogue liraglutide, GFRAL-deficient mice remain responsive to GLP-1 and leptin, whereas GLP-1 and leptin knock-out mice are responsive to GDF15 administration[39]. Furthermore, in studies in human volunteers, neither GLP-1 nor PYY infusion altered GDF15 levels[26].

Considering the evidence linking GDF15 to nutritional stress, the elevations in GDF15 seen in people with obesity with and without T2DM, may in fact represent a counter-regulatory mechanism aiming to reduce an increased circulating fatty acid load[27]. Consequently, the reduction in GDF15 that was observed in this study may represent a state of resolving metabolic/nutritional stress. It is also plausible that the physiological effect of GDF15 secretion may depend on the conditions this is secreted under, as is the case for other cytokines[66]. Further studies on human participants are however going to be required in order to fully understand the links between bariatric surgery and GDF15, as well as their role in eating behaviour following bariatric surgery.

### 4.5.5 Conclusion

Our data are in line with the relevant literature and suggest GDF15 does not act as a signal of energy availability, nor does it respond to changes in nutrient availability, following fasting and/or eating. However, our finding of an acute change in salivary GDF15 following exposure to fatty acids, suggests that GDF15 has a role in lipid signalling and potentially the homeostasis of lipid metabolism. Finally, our data from our bariatric surgery participants in Study 4, illustrate that changes in GDF15 secretion following bariatric surgery have a differential relationship to post-operative %WL in people with and without T2DM. This study for the first time illustrates a relationship
between a reduction in GDF15 and post-operative weight loss, which may represent the resolution of an obesity-associated state of nutritional stress.
4.6 Chapter 4 References


Appendix 4.1: Labelled magnitude scale

Subject Number

Date

Most liked sensation imaginable

Like extremely

Like very much

Like moderately

Like slightly

Dislike slightly

Dislike moderately

Dislike very much

Dislike extremely

Most disliked sensation imaginable

Please rate how strong you found the taste by putting a cross on the scale above
Appendix 4.2: Taste questionnaire

Date........................................... Participant ID...........................................

Taste and smell questionnaire

Please circle the most appropriate answer or circle the relevant number on the scale (1-10)

Q1. Have you noticed any change in your appetite since your weight loss surgery?

YES/ NO

Q2. Have you noticed any change in the taste of food or drink since your weight loss surgery?

YES/ NO

Q3. Have you noticed any change in your sense of smell since your weight loss surgery?

YES/ NO

Q4. Have you experienced an overall loss of taste since your weight loss surgery?

YES/ NO

If you answered YES to ANY OF the above questions please continue. If you answered NO to ALL of the above questions, do not proceed any further. Please return the questionnaire.
Q5. If you have had a loss of taste, is that loss:

<table>
<thead>
<tr>
<th>No loss</th>
<th>Partial</th>
<th>Complete</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>4</td>
<td>5</td>
<td>6</td>
</tr>
<tr>
<td>7</td>
<td>8</td>
<td>9</td>
</tr>
<tr>
<td>10</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Q6. Are there any foods that are repulsive or intolerable to you since your weight loss surgery?

YES/ NO

If you answered yes then please state what:

Q7. Are there any foods that taste different to you since your weight loss surgery?

YES/ NO

If yes, please state what food:

Q8. Has your overall taste increased in intensity since your weight loss surgery?

YES/ NO

Q9. Have you experienced an increase in taste for sweet foods?

YES/ NO

Q10. Have you experienced a decrease in taste for sweet foods?

YES/ NO

Q11. If you have had a loss of sweet taste, is that loss:
### Q12. Have you experienced an increase in taste for salty foods?

**YES/ NO**

### Q13. Have you experienced a decrease in taste for salty foods?

**YES/ NO**

### Q14. If you have had a loss of salty taste, is that loss:

<table>
<thead>
<tr>
<th>No loss</th>
<th>Partial</th>
<th>Complete</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>4</td>
<td>5</td>
<td>6</td>
</tr>
<tr>
<td>7</td>
<td>8</td>
<td>9</td>
</tr>
<tr>
<td>10</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### Q15. Have you experienced an increase in taste for sour foods?

**YES/ NO**

### Q16. Have you experienced a decrease in taste for sour foods?

**YES/ NO**

### Q17. If you have had a loss of sour taste, is that loss:

<table>
<thead>
<tr>
<th>No loss</th>
<th>Partial</th>
<th>Complete</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Q18. Has this change in taste affected how much you eat?

YES/ NO

Q19. In your opinion, has your change in taste affected your weight loss?

YES/ NO

Q20. Overall, do you feel that your taste has increased or decreased in intensity since your weight loss surgery?

Increased/ Decreased

Q21. In your opinion, does loss of taste lead to better weight loss?

YES/ NO

Q22. Is your postoperative change in taste greater or less than what you expected preoperatively?

Greater/ Less

Q23. How important is taste to the enjoyment of food?

Important/ Not important

Q24. Have you experienced an overall loss in your sense of smell since your weight loss surgery?
Q25. If you have had a loss of smell, is that loss:

No loss | Partial | Complete
---|---|---
1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10

Q26. Are there any foods that smell differently to you since your weight loss surgery?

YES/ NO

If yes, please state what food?

Q27. In your opinion, has your change in smell affected your weight loss?

YES/ NO

Q28. Overall, do you feel that your smell has increased or decreased in intensity since your weight loss surgery?

Increased/ Decreased

Q29. Is your postoperative change in smell greater or less than what you expected preoperatively?

Greater/ Less

Q30. How important is smell to the enjoyment of food?

Important/ Not important

Q31. Do you eat less food because it does not taste or smell good?
YES/ NO

Q32. Do you eat less food because you are simply not hungry?

YES/ NO

Q33. Is your postoperative change in appetite greater or less than what you expected preoperatively?

Greater/ Less

Thank you!
5. Chapter 5: TASTER Study

5.1 Introduction

The use of brain imaging has become increasingly important in providing insights into the pathophysiology of obesity and potential therapeutic targets within the CNS regions that control appetite. Neuroimaging studies conducted in people living with overweight and obesity, as well as in people who underwent bariatric surgery, have significantly improved our understanding of the central control of eating behaviour, how this is altered in people with obesity and, importantly, the eating behaviour changes seen following bariatric surgery[1]. Functional magnetic resonance imaging (fMRI) is a type of imaging aimed at demonstrating real-time, regional changes in brain metabolism[2]. Activity within a brain region results in increased oxygen demands, which in turn leads to a compensatory increase in regional blood flow, increasing the oxygenated haemoglobin in the regional blood supply[2].fMRI thereby enables the measurement of changes in event-related transient neural activity, through measuring blood-oxygen-level dependent (BOLD) contrast (Figure 5.1)[3].
Figure 5.1: Schematic diagram illustrating brain tissue in resting and stimulated state

Figure 5.1: When a region of the brain is activated by an event or action, the increased neuronal activity results in an increased local energy requirement, which leads to up-regulation of the cerebral metabolic rate of oxygen. The increased oxygen consumption results in a compensatory increase in regional blood flow, which results in increased levels of oxygenated haemoglobin in the regional blood supply[2]. Thereby, an increase in regional brain activity can be detected on fMRI by measuring BOLD contrast[3]. BOLD: Blood-oxygen-level dependent contrast. (Figure from [2])

The cognate receptors for multiple gut hormones are present throughout the CNS, both in regions involved in the homeostatic control of appetite and body weight, but also in reward-related regions[4,5]. fMRI studies have demonstrated that gut hormones including GLP-1, PYY and ghrelin, directly modulate activity in these centres[5]. An overview of the main CNS regions involved in eating behaviour is provided in Table 5.1. Following a meal, PYY and GLP-1 synergistically modulate activity in the brain’s appetitive network and reduce the reward value of food[5]. A negative correlation has been demonstrated between PYY levels and activity in the brain’s reward centres[5]. A study comparing the effects of a meal to intravenous PYY and GLP-1 administration, both separately and in combination, demonstrated the effects PYY and GLP-1 on the CNS appetitive network mimicked the fed state[6].
Table 5.1: Central nervous system areas involved in the control of appetite and eating

<table>
<thead>
<tr>
<th>Brain Area</th>
<th>Role in eating behaviour</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hypothalamus</td>
<td>Homeostatic control</td>
</tr>
<tr>
<td>Hippocampus</td>
<td>Learning and memory</td>
</tr>
<tr>
<td></td>
<td>Links energy balance to incentive behaviour</td>
</tr>
<tr>
<td>Amygdala</td>
<td>Emotional learning, assigning of value</td>
</tr>
<tr>
<td></td>
<td>Encodes palatability of foods</td>
</tr>
<tr>
<td></td>
<td>Link between homeostatic and hedonic interactions</td>
</tr>
<tr>
<td>Insula (“Ingestive” and “gustatory” cortex)</td>
<td>Encodes sensory information of taste, integrated with sensory cues to form flavour</td>
</tr>
<tr>
<td></td>
<td>Responds to food cues and gut hormones</td>
</tr>
<tr>
<td>Nucleus of the solitary tract</td>
<td>Afferent target for vagus nerve</td>
</tr>
<tr>
<td>Ventral tegmental area</td>
<td>Encodes nutritional and reward values of food</td>
</tr>
<tr>
<td></td>
<td>Generated value and motivational signals</td>
</tr>
<tr>
<td>Cerebellum</td>
<td>Integrates and coordinates somatic-visceral responses to food</td>
</tr>
<tr>
<td>Striatum:</td>
<td></td>
</tr>
<tr>
<td>Caudate nucleus</td>
<td>Encodes motivational and incentive properties of food</td>
</tr>
<tr>
<td></td>
<td>Anticipatory reward</td>
</tr>
<tr>
<td>Putamen</td>
<td>Connected to all other brain regions of eating behaviour</td>
</tr>
<tr>
<td>Nucleus accumbens</td>
<td>Links motivation to action</td>
</tr>
<tr>
<td>Orbitofrontal cortex</td>
<td>Reward encoding</td>
</tr>
<tr>
<td></td>
<td>Integration of sensory, cognitive and reward aspects</td>
</tr>
<tr>
<td>Cingulate cortex</td>
<td>Decision making</td>
</tr>
<tr>
<td>Prefrontal cortex</td>
<td>Translation of internal &amp; external cues to behaviour, food-related decision-making</td>
</tr>
</tbody>
</table>

Table adapted from[1]

In line with the finding that people with obesity consistently rate energy-dense foods as more palatable compared to normal weight individuals, fMRI studies demonstrate higher reward responses to energy-dense foods in people with obesity[7-10]. Furthermore, in people with obesity the degree of BOLD activation in response to visual food cues displays a positive correlation to increasing BMI[11]. A number of fMRI studies have also investigated reward responses to gustatory stimuli and have shown
stronger reward responses to taste in people with obesity[12-14]. Stronger activation compared to lean individuals has been detected in both cortical (anterior cingulate cortex, insular and opercular cortices, orbitofrontal cortex) and subcortical (amygdala, nucleus accumbens, putamen and pallidum) areas[15]. The fMRI literature suggests that increased food-cue reactivity may be a driver for high energy intake in people with obesity. Consequently, altered metabolic and neurological responses to food predispose individuals with obesity to further weight gain[10].

A series of fMRI studies have provided evidence surrounding the neural correlates of the eating behavior changes induced by bariatric surgery. The increased food-cue responsivity associated with obesity resolves following bariatric surgery[16-18]. A study by Li et al., correlated a reduction in visual food-cue reactivity with a post-operative reduction in circulating ghrelin levels[19]. Interestingly, in a study administering octreotide, a somatostatin analogue with widespread endocrine inhibitory effects, during fMRI scanning in people who had a RYGB resulted in increased food-related reward responses and reward-area BOLD activity correlated with octreotide-induced reductions in circulating PYY[20]. A further study utilised GLP-1 receptor blockade in participants post-RYGB who consumed a milkshake during imaging and demonstrated that GLP-1 receptor blockade resulted in increased insular activation[21]. These findings, taken together, suggest a role for PYY and GLP-1 in reducing the reward response to energy-dense foods following bariatric surgery. To date only one study has directly compared the effect of RYGB with SG with regard to their effect on the gustatory system and demonstrated associations between a post-operative reduction in taste-induced activation and weight loss post-RYGB but not following SG[22]. The exact effects of SG, the most commonly performed bariatric procedure, and taste function remains incompletely understood. Furthermore, no weight-loss matched studies have compared the effects of SG to diet-induced weight using low-calorie or meal replacement diets. Improving our understanding of the mechanisms by which SG leads to altered appetite and taste and in particular the biological drivers of these changes will enhance our understanding of why bariatric surgery is so successful and potentially lead to the development of non-surgical approaches to treat obesity.
5.2 The TASTE and eating behaviour (TASTER) Study

The TASTER Study is an observational study, designed to gain insight into brain structure, connectivity and the neural networks that control appetite and taste in people living with severe obesity and following weight loss, either through SG or a meal replacement, as well as in people with healthy weight using MRI and fMRI.

The study aimed to compare the effects of 10% WL following SG, on brain circuits that control energy intake and taste, brain structure and connectivity to the effects of 10% weight loss induced by a lifestyle intervention, in order to differentiate the effects of SG and the effects of weight reduction per se. The study was aiming to investigate responses in brain circuits that control energy intake and taste, brain structure and connectivity, as well as gut-derived hormone and adipokine levels. The study aimed to compare these in people with severe obesity, following SG, following a lifestyle intervention and in people with a healthy weight. Based on a power estimation taking into account findings from the existing fMRI literature, the study planned to recruit 75 participants into 3 study groups (25 per group):

- **SG group:**
  - BMI of 35-50 kg/m²
  - Planned to undergo primary SG and fulfilling eligibility criteria for bariatric surgery.

- **Lifestyle intervention group:**
  - BMI of 35-50 kg/m² (age, sex and BMI matched to the SG group)
  - Willing to undergo a lifestyle intervention programme, delivered through the study, with a view to achieving approximately 10% weight loss.

- **Normal weight group:**
  - BMI 18.5-24.9 kg/m² (age and sex matched to the SG and lifestyle intervention groups).
The TASTER study was intended to form the main body of work for this thesis. Work on designing the protocol commenced in October 2017. Upon completion of the preliminary version of the protocol, a patient focus group was arranged with patients attending the Tier 3 weight management programme at the UCLH Bariatric Centre for Weight Management and Bariatric surgery to receive feedback on the acceptability of the study protocol and the design of the lifestyle intervention. In order to decide on a taste delivery approach, a visit was carried out in December 2017 to the Interdisciplinary Centre for Smell and Taste, The University of Dresden, Card Gustav Carus University Hospital, Dresden, Germany (https://www.uniklinikum-dresden.de/de/das-klinikum/kliniken-polikliniken-institute/hno/forschung/interdisziplinaeres-zentrum-fuer-riechen-und-schmecken/homepage-in-englisch-1), a clinical and research centre with expertise in the assessment of patients with disorders of smell and taste and multi-modality research methodologies for assessing olfactory and gustatory function in clinical research studies. This visit provided an opportunity to experience different assessment techniques for smell and taste function, but also to gain insights into the type of data they generate as well as their limitations. Subsequently, the taste delivery system used in the TASTER study was conceptualised and designed based on a previous version of an MRI taste-delivery device, the “MR Fluid Liquid Automatic Vendor” and was built by the Engineering Section of the Centre for Neuroimaging Sciences, King’s College London (KCL) Institute of Psychiatry, Psychology and Neuroscience(Figure 5.2). The taste stimuli used in the study were chosen after a focus group blindly evaluated several different options of chocolate milkshakes and varying concentrations of glucose solutions. The full protocol of the Taster Study can be seen in Appendix 1. The study received Ethical Approval from the West London HRA Research Ethics Committee in August 2018 and opened to recruitment in March 2019. Study visits were carried out at the King’s College Hospitals (KCH) Clinical Research Facility. By March 2020, 30 participants were recruited into the study, 8 in SG group, 12 in the lifestyle intervention group and 8 in the healthy weight group. In March 2020, following the outbreak of the COVID-19 pandemic, the TASTER Study was suspended. Participants who were on a meal replacement diet at this time were followed up remotely and were offered the option of either continuing the diet plan until the target %WL was achieved or following
a food re-introduction plan. Follow-up was subsequently arranged with the referring clinical teams. Due to restrictions that remained in place at KCH in line with their COVID-19 regulations and the need to prioritise COVID-19 related research the study was unable to reopen. Due to the complexities of the imaging protocol and the in-scanner taste delivery system, as well as geographical restrictions, it was not possible to relocate the study to a different MRI imaging unit. Following a 15-month interruption to study related activity it was deemed that it was no longer possible to re-open the study as originally planned, and it was discontinued. The data collected up until March 2020 allowed for a comparison of taste-related activity in the appetitive network in people with obesity compared to people of normal weight in the fasted and the fed state and the findings from this analysis are presented in this chapter.

Figure 5.2: The taste delivery system used in the TASTER Study

Figure 5.2 illustrating the taste delivery system used in the TASTER study, which is an automated liquid delivery system that dispenses calibrated bolus of 0.3mL for each of the three tastes used in the study (water, sucrose and chocolate milkshake).
5.3 TASTER Study: Summary of the work presented in this chapter

The present part of the study compared the neurological responses to taste in individuals with severe obesity (BMI 35-50kg/m²) aged 18-50 years and a group of age-matched adults with normal weight (BMI 18.5-24.9kg/m²) in the fed and fasted state. fMRI imaging was performed and BOLD signal measured while participants tasted three different foods during the scans:

- Sucrose solution: to assess responses to a sweet stimulus.
- Chocolate milkshake: to assess responses to a high-fat sweet stimulus (the sugar content of the sucrose solution was matched to the chocolate milkshake).
- Water: as a neutral stimulus known to activate the gustatory cortex
- Control condition: Imaging was obtained but no taste stimulus delivered.

5.3.1 Hypotheses

The work presented in this chapter attempted to evaluate the following hypotheses:

- Severe obesity leads to modulation of neural activity and connectivity within the CNS circuits that control the perception and reward value of taste
- Severe obesity leads to an increased reward value toward sweet and fat-containing taste stimuli
- In individuals with normal weight, the reward value of the taste of sweet and fat-containing stimuli does not differ
- In individuals with severe obesity, the fed condition does not lead to a reduced reward value to sweet and fat-containing taste stimuli compared to the fasted state

5.3.2 Methods

5.3.2.1 Participants and recruitment

Participants for the severe obesity group were recruited from the UCLH Bariatric Centre for Weight Management and Metabolic Surgery, the Department of Bariatric and Metabolic Surgery at University Hospital Lewisham (UHL) and the Department of
Bariatric Surgery at KCH. Participants for the normal weight control group were recruited from UCL and KCL. The study received ethical approval (IRAS 238390, protocol number 18/0233) and was carried out in line with the Declaration of Helsinki and following the standards of Good Clinical Practice. Participation was voluntary and written informed consent was obtained from participants. Potentially eligible participants were first approached by a member of the clinical team and if interested, they were provided with a copy of the Participant Information Sheet. They were allowed time to consider this information and ask questions. If they opted to participate, written informed consent was obtained through signing a consent form.

The inclusion criteria were a BMI 35-50 kg/m² for participants in the severe obesity group and 18.5-24.9 kg/m² for participants in the normal weight control group. Additional inclusion criteria were age between 18-50 years, being right-handed and understanding written and spoken English. The exclusion criteria were:

- Type 1 or type 2 diabetes mellitus.
- Chronic neurological conditions resulting in an abnormal MRI scan.
- Any acute or chronic condition impairing an individual’s sense to taste or smell.
- Contraindications specific to MRI scanning: metal implants, aneurysm clips, cochlear implants, exposure to metal flakes/splinters, body weight above the limit of the MRI scanner (150kg).
- Difficulties lying flat for more than 1 hour.
- Menopause (in view of the finding that menopause affects gustatory function).
- Current smoking.
- The use of medication that impact upon weight.
- Pregnancy or lactation.
- Untreated B12 or zinc deficiency (in view of the finding that menopause affects gustatory function).

5.3.2.2 Study visit assessments
Participants initially attended the KCH Clinical Research Facility for a screening and acclimatisation visit where their eligibility to participate in the study was confirmed.
During the visit, a medical and drug history were obtained, the participants’ height and weight were recorded, and the participants were asked to try all the study foods and the taste liquids used during imaging and rate how much they like them on a labelled magnitude scale to ensure they did not dislike any of these. Screening blood samples were obtained to measure HbA1c, B12, zinc and in females with irregular menstrual cycles, follicle-stimulating hormone (FSH) and luteinising hormone (LH). Participants also completed an MRI safety questionnaire at every visit. In addition, a screening structural MRI scan was performed. This ensured that any structural brain lesions could be excluded prior to the first scanning visit and that participants were able to tolerate MRI scanning. At the end of the scan, the participants performed a practice run of the taste task inside the MRI scanner but without being scanned at the time, to ensure familiarity with the process during their subsequent scanning visit.

Participants who were deemed eligible to enrol following this visit were booked in for two study visits, at least 48h and up to 7 days apart, and were randomly allocated to be studied fed or fasted first. Participants were instructed to avoid alcohol and strenuous physical activity on the day preceding their study visits and to fast overnight for a 12-hour period. Study visits were booked in the morning (8:30-10:30 am) and participants were advised not to consume caffeine on the morning of their visit and to only drink water. Participants arrived fasted and an intravenous cannula was inserted into a vein on their arm. They were allowed to rest for approximately 45 minutes. The date of the last menstrual period was recorded for female participants and a urinary pregnancy test was performed. On fasted study visit days, participants were taken to the scanning room following the rest period and the MRI scan was performed. On fed visit study days, following the rest period, participants were given a 500kcal liquid breakfast meal (Resource fibre plus, vanilla flavour, 250mL). One hour following the meal, participants were taken to the scanning room and the MRI scan was performed.

5.3.2.3 MRI scanning protocol

The MRI scanning protocol included structural imaging, diffusion tensor imaging, resting state fMRI and task-based fMRI. Structural imaging was obtained, and high-resolution anatomical images acquired through a three-dimensional magnetisation-
prepared rapid gradient echo T1-weighted sequence. Images for conventional T2-weighted and FLAIR were also acquired. Brain responses to 4 taste conditions (sweet taste, sweet/fatty taste, water, no taste), delivered during fMRI imaging were measured via image acquisition for BOLD. Boluses of the 3 tasting liquids (sucrose solution, chocolate milkshake, water) were delivered following a visual cue in a randomised order to the participants. Participants were given visual commands displayed on a screen to initially keep the liquid in their mouth and taste it and subsequently to swallow. Each task block lasted 7 minutes and was performed twice.

5.3.2.4 In-scan taste delivery system
The taste delivery system used in the study is an automated liquid delivery system that dispenses a calibrated bolus of 0.3mL. The bolus volume is set to 0.3mL to ensure easy swallowing in the supine position. The taste delivery system is made up of three constituent parts: 1) the Pneumatic control device, which is situated in the scanner control room, 2) the dispensing containing 4 liquid reservoirs and calibrated dose syringes and 3) a tube which extends from the main unit to delivery device which is attached to the head coil inside the scanner. The pneumatic control device conveys the paradigm commands via a pneumatic programmed sequence. The device receives air inflow and pumps out pressurised air along a 4m piping tube to the dispensing unit. Once triggered via a command from the paradigm, the respective calibrated syringe is pneumatically pushed from the pressurised air and delivers a bolus along the delivery tube. The syringe mechanism subsequently retracts via pneumatic suction which draws up the next bolus from the reservoir. The delivery tube ends in a solid mouthpiece with three outlets, one for each of the three tastes that were used in the study, avoiding cross-contamination between the tastes. The mouthpiece is attached to the head coil through a movable holding device and is positioned above the participant’s mouth during scanning. Disposable single used covers were used during scanning and changed between participants.

5.3.2.5 Taste solutions delivered during scanning
The following tastes were delivered during scanning in order to assess BOLD responses:
• Chocolate milkshake: to assess responses to a high-fat sweet stimulus (Ensure plus chocolate milkshake, Abbott Nutrition).
• Sucrose solution: to assess responses to a sweet stimulus. A sucrose solution of 0.4M was used, which was calculated to match the sugar content of the chocolate milkshake.
• Water: as a neutral stimulus known to activate the gustatory cortex (bottled mineral water, Highland springs).
• Control condition: Imaging was obtained but no taste stimulus delivered.

5.3.2.6 Image acquisition
MRI scanning was performed on MR750 3 Tesla GE Discovery Scanner (General Electric, Waukesha, WI, United States of America) with a 32-channel receive-only head coil. Heart rate and oxygen saturations were monitored during scanning using a pulse oximeter placed on the index or middle finger of the left hand. Whole brain functional images were acquired using a single-shot 2D T2* weighted gradient echo EPI sequence using parallel imaging (Array coil Spatial Sensitivity Encoding). Slices were acquired in a sequential top-down direction in the near-axial plane parallel to the anterior-posterior commissure line (approximately 30) with the following parameters: Repetition Time (TR) = 200 ms, Echo Time (TE) = 30ms, flip angle = 75, matrix size = 64 x 64, FOV = 211 x 211, slice thickness = 3.3 mm, slice gap = 0.3 mm, no. of slices = 41, in-plane voxel size = 3.3 x 3.3 mm². High resolution structural T1-weighted imaging was acquired for each subject, using a T1 weighted 3D Magnetisation Prepared Rapid Acquisition Gradient Recalled Echo sequence with the following parameters: slice thickness (s)=1.2mm, slices = 196, TR = 7.312ms, TE = 3.016ms, inversion time (TI) = 400ms, flip angle (FA) = 11°, matrix size (DM) = 256x256 with a FOV = 27cm. Acquisition time: 5:37 min.

5.3.2.7 Structural image processing
T1 weighted images obtained from all study participants were used to create a group template using Advanced Normalisation Tools [23,24], registered to standardised MNI
space using the FLIRT routine of the Functional Software Library (FSL, FMRIB, Oxford University, version 3.2)\(^{[25,26]}\).

### 5.3.2.8 Functional image processing

Image processing was carried out by a neuroimaging researcher and was performed using a combination of neuroimaging software packages. For motion correction, timeseries images were realigned to a base volume using 3dVolreg (AFNI) and all volumes were corrected for any slice-timing differences using 3dTshift (AFNI). A mean timeseries image was calculated (3dTstat) and co-registered to the subject specific T1 anatomical image (using FSL, FMRIB, version 3.2, University of Oxford, UK, \(\text{http://www.fmrib.ox.ac.uk/fsl}\)). Warping parameters for the anatomical to standard MNI template were subsequently applied using Advanced Normalisation Tools\(^{[23,24]}\).

Spatial filtering of the images was carried out using a full width half maximum gaussian kernel of 8 x 8 x 8 mm using the Statistical Parametric Mapping smoothing function (SPM-12, Wellcome Trust Centre for Neuroimaging, University College London, UK, \(\text{http://www.fil.ion.ucl.ac.uk/spm}\)). A high-pass temporal filter with a width of 128s was then applied as part of the first level model design.

### 5.3.2.9 Statistical analysis

Descriptive statistics were carried out using SPPS version 25 to describe the study population. Continuous measures were summarised using means and SD. Categorical measures were summarised using proportions. Parametric t-tests, one-way and two-way ANOVA and non-parametric tests were used as appropriate. The significance level was set as \(p<0.05\).

For statistical analysis of whole brain data was performed using the SPM-12 package. A random effects analysis was implemented by creating 1\(^{\text{st}}\) level contrast images from a standard general linear model analysis. These contrast images were taken through to 2\(^{\text{nd}}\) level to analysis in order to investigate potential group level effects. The paradigm was modelled as an event-related task with condition onsets and durations defined from the task. The duration of the cue presentation was set to 1.5s and the stimulus delivery was set to 3s. The time from the stimulus being delivered until the stimulus
was swallowed varied randomly across the task (4.5s to 7.5s). Each condition was modelled separately for each of the three taste stimuli used, as well as the control condition (no taste delivery). In addition, the 1.5s swallowing periods were modelled with onsets defined as the time swallow cue was presented. The swallow cue was modelled as a single condition, as opposed to a taste specific condition. Each condition was convolved with SPM’s canonical haemodynamic response function[27]. Furthermore, the six ‘scan-to-scan’ affine head motion parameters produced during motion correction were included as nuisance regressors in the model to account for participants’ head movements. Linear contrasts were generated to examine the differences in BOLD response between conditions. Linear contrasts were replicated for both runs of the taste task and a single contrast statistical parametric map was subsequently created for each of the contrasts.

Contrast maps created at the first level included:

- Contrast 1: Water > No taste
- Contrast 2: Sweet taste (sucrose) > No taste
- Contrast 3: Chocolate milkshake > No taste
- Contrast 4: Sweet taste (sucrose) > Water taste
- Contrast 5: Chocolate milkshake > Water taste
- Contrast 6: Chocolate milkshake > Sweet taste (sucrose)

Random effects second level models were created to investigate differences between the two groups, the fed versus fasted condition and any interaction effects. The contrast maps created at the 1st level were entered into individual full factorial models, with the factor defined by study group (participants with obesity vs. healthy weight participants) and study condition (fasted vs. fed). Whole brain statistical analysis was carried out using a cluster defining threshold of p<0.001 uncorrected. The significance level for clusters was set at p<0.05 following a family wise error correction for multiple voxel comparisons, using the SPM cluster extent threshold definition.
5.3.3 Results

5.3.3.1 Participants
Enrolled participants who completed the baseline visits were included. Two participants were excluded from the analysis, one because they only attended for one of the two baseline visits and the other participant due to an image artefact in the functional image sequences. Hence, a total of 17 participants with severe obesity and 8 participants of healthy weight were included in the analysis. Results were therefore treated as exploratory. Mean BMI in the group of participants with severe obesity was 41.0kg/m$^2$ ±4.3. Due to COVID-19 restrictions it was not possible to access the study notes for participants in the healthy weight group at the time of writing, hence their mean BMI was not calculated and ethnicity is not stated, however all participants had a BMI between 18.5–24.9kg/m$^2$. Participant demographics can be seen in Table 5.2. The two study groups were comparable in terms of age and sex of recruited participants.

<table>
<thead>
<tr>
<th>Table 5.2: Participant demographics</th>
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<tr>
<td><strong>Severe obesity</strong> (n=17)</td>
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<tr>
<td>Age</td>
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<tr>
<td>Sex</td>
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</tbody>
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5.3.3.2 Whole brain analysis
Whole-brain BOLD responses were analysed for participants in both of the study groups for each of the two study conditions and the 8 contrasts generated by comparing the responses between the tastes delivered during scanning. Whole brain analysis results are presented by contrast. For the purpose of describing the main effects of taste stimulation statistical maps will be presented before describing the main statistical differences. In view of the very strong taste response effect in some regions and for illustrative purposes, a high threshold has been used for some of the parametric maps to allow for differentiation of smaller brain regions.
5.3.3.3 Contrast 1: Taste of water compared to no taste

The BOLD amplitude upon tasting water compared to not receiving any stimulus was significant across multiple regions of the brain associated with taste perception (Figure 5.3). In participants with obesity, the taste of water produced strong BOLD responses originating from the bilateral anterior insula, frontal operculum, caudate, putamen, supplementary motor area, dorsal lateral prefrontal cortex, as well as the visual cortex (Figure 5.3A). Similarly, in participants with healthy weight, the taste of water generated strong BOLD responses originating from the bilateral anterior insula, frontal operculum, caudate, putamen, supplementary motor area, dorsal lateral prefrontal cortex, as well as the visual cortex (Figure 5.3B). Statistical analysis revealed no significant differences between participants with obesity compared to participants with healthy weight, nor between the fed and fasted state and no interactions.

Figure 5.3: Whole-brain BOLD responses to water compared to no taste in participants with obesity compared to participants with healthy weight

Figure 5.3 illustrating whole-brain BOLD responses to water compared to no taste in participants with obesity (A) compared to participants with healthy weight (B). The BOLD amplitude upon tasting water compared to no stimulus was significant across multiple regions of the brain. Statistical analysis revealed no significant differences between participants with obesity compared to participants with healthy weight, nor between the fed and fasted state and no interactions. BOLD: Blood-Oxygen-Level Dependent.
5.3.3.4 Contrast 2: Sweet taste (sucrose) compared to no taste

Similar to water, the BOLD amplitude upon receiving a sweet taste (sucrose solution) compared to not receiving any stimulus was significant across multiple CNS regions associated with taste perception (Figure 5.4A). In participants with obesity, sucrose generated strong BOLD responses in the anterior insula bilaterally, the frontal operculum, caudate, putamen, supplementary motor area, dorsal lateral prefrontal cortex as well as the visual cortex. The taste of the sucrose solution generated strong BOLD responses originating in the same regions in participants with a healthy weight (Figure 5.4B).

Between group comparison identified a significant cluster in the left dorsal lateral prefrontal cortex ($p=0.01$, 756 voxels in size, $T=5.01$, peak voxel MNI co-ordinates $-44\ 28\ 38,\ -38\ 24\ 48,\ -8\ 52\ 52$). This result describes a greater BOLD response in participants with obesity compared to participants with healthy weight (Figure 5.4C). A post-hoc analysis did not reveal any significant clusters between groups for either the fasted or fed condition. There were no interactions found from this contrast.

Figure 5.4: Whole-brain BOLD responses to sweet taste (sucrose) compared to no taste in participants with obesity compared to participants with healthy weight

![Figure 5.4](image-url)

Figure 5.4: illustrating whole-brain BOLD responses to sucrose (sweet taste) compared to no stimulus in participants with obesity (A) compared to participants with healthy weight (B) and the significant difference between the groups (C). The taste of the sucrose solution generated BOLD responses originating in the same CNS regions in participants with a healthy weight and with obesity. A significantly greater BOLD response in participants with obesity was observed, compared to participants with healthy weight, in the left dorsal lateral prefrontal cortex (C). BOLD: Blood-Oxygen-Level Dependent.
5.3.3.5 Contrast 3: Chocolate milkshake compared to no taste

Similar to water and the sucrose solution, the BOLD amplitude upon tasting the chocolate milkshake compared to no taste stimulus was significant across multiple CNS regions associated with taste perception. Tasting the chocolate milkshake generated strong BOLD responses originating from the bilateral anterior insula, frontal operculum, caudate, putamen, supplementary motor area, dorsal lateral prefrontal cortex as well as the visual cortex both in participants with obesity (Figure 5.5A) and in participants with a healthy weight (5.5B).

When comparing between groups, a significant cluster was identified in the left dorsal lateral prefrontal cortex ($p=0.02$, 612 voxels in size, $T=5.14$, peak voxel MNI coordinates $-38 38 22$, $-44 28 38$, $-34 24 38$). This result describes a greater response in participants with obesity compared to participants with a healthy weight (Figure 5.5C). A post hoc analysis did not reveal any significant clusters between groups for either the fasted or fed state. There were no differences identified between the fed and fasted state and no interactions from this contrast.

Figure 5.5: Whole-brain BOLD responses to chocolate milkshake compared to no taste in participants with obesity compared to participants with healthy weight

![Figure 5.5](image)

Figure 5.5 illustrating whole-brain BOLD responses to chocolate milkshake compared to no taste in participants with obesity (A) compared to participants with healthy weight (B) and the significant difference between the groups (C). The taste of the chocolate milkshake solution generated BOLD responses originating in the same CNS regions in participants with a healthy weight and with obesity. A significantly greater BOLD response in participants with obesity was observed, compared to participants with healthy weight, in the left dorsal lateral prefrontal cortex (C). BOLD: Blood-Oxygen-Level Dependent.
5.3.3.6 Contrast 4: Sweet taste compared to water

In this contrast, the effect of tasting the sweet sucrose solution was compared to the effect of tasting water. Comparing the BOLD response from tasting the sucrose solution to tasting water did not reveal any significant clusters. However, a significant group effect was identified in participants with obesity compared to participants with healthy weight, which provided a significant cluster encompassing parts of the anterior insula and frontal operculum (p=0.003, 1018 voxels in size, T=4.31, peak voxel MNI co-ordinates -32 0 -4, -30 -8 2, -54 14 -4) (Figure 5.6A). Post-hoc analysis did not show any differences relating to the fasted versus the fed state and no significant interactions were identified.

5.3.3.7 Contrast 5: Chocolate milkshake compared to water

In this contrast, the effect of tasting the chocolate milkshake was compared to the effect of tasting water. Comparing the BOLD response from tasting the chocolate milkshake to the response from tasting water did not reveal any significant clusters in the healthy weight participants. However, a significant cluster in the left primary motor cortex was identified in participants with obesity (p=0.03, 545 voxels in size, T=4.38, peak voxel MNI co-ordinates -60 -8 22, -52 -8 26, -56 -8 42).

A significant group effect was identified in participants with obesity compared to healthy weight participants, which provided a significant cluster encompassing parts of the anterior insula and frontal operculum (p=0.029, 525 voxels in size, T=3.97, peak voxel MNI co-ordinates -60 -8 22, -52 -8 26, -56 -8 42) (Figure 5.6B). Post-hoc analysis did not provide any significant results when comparing between groups in the fasted versus the fed condition.
Figure 5.6: Whole-brain BOLD responses clusters in participants with obesity compared to participants with healthy weight in response to tasting the sucrose solution (A) and chocolate milkshake (B) compared to tasting water.

Figure 5.6 illustrating whole-brain BOLD responses clusters in participants with obesity compared to participants with healthy weight in response to tasting the sucrose solution (A) and chocolate milkshake (B) compared to tasting water. A significant cluster in the left primary motor cortex was identified in participants with obesity. In participants with obesity compared to healthy weight participants, a significantly greater BOLD response was observed encompassing parts of the anterior insula and frontal operculum (B). BOLD: Blood-Oxygen-Level Dependent.

Comparison between the fasted and fed state within groups, showed a significant effect relating to the fasted state in participants with obesity. This result was identified as a greater response in the fasted compared to the fed state and provided a significant cluster within a region of the anterior cingulate cortex (p=0.03, 517 voxels in size, T=4.98, peak voxel MNI co-ordinates -6 30 12, -14 36 14, 8 32 12) (Figure 5.7). No condition effects in either direction were identified in healthy weight participants and no interaction effects were found.
Figure 5.7: Difference in whole-brain BOLD responses to chocolate milkshake compared to water in participants with obesity in the fasted versus the fed state.

Figure 5.7 illustrating the difference in whole-brain BOLD responses to chocolate milkshake compared to water in participants with obesity in the fasted versus the fed state. In participants with obesity, a significantly greater BOLD response within the region of the anterior cingulate cortex was observed in the fasted compared to the fed state. BOLD: Blood-Oxygen-Level Dependent.

5.3.3.8 Contrast 6: Chocolate milkshake compared to sweet taste (sucrose)
Direct comparisons between the taste of the chocolate milkshake and the taste of the sucrose solution revealed no significant clusters between groups and no effects of fed versus fasted state.

5.3.3.9 ROI analysis
The BOLD response for each of the contrasts identified were also compared between groups and for the fasted versus fed condition in a series of ROI relevant to taste perception and food-related reward:

**Anterior cingulate cortex:** No significant differences in BOLD signal were identified in any of the contrasts between the different tastes in participants with obesity. In participants with healthy weight a significant difference was identified in Contrast 1, comparing the taste response to water, compared to no taste, indicating a higher
response in the fed state (Water > No taste 0.589 ±0.452 fasted vs 0.886 ±0.336 fed, p=0.026). No differences were seen between participants with obesity and participants with healthy weight in the fasted nor the fed state.

**Nucleus accumbens:** No significant differences in BOLD signal were detected between the fasted and fed state in participants with obesity nor in participants with healthy weight for any of the contrasts. In the fasted state a significant difference was noted between participants with obesity and participants of healthy weight in the contrast comparing BOLD signal to sucrose compared to water, with higher BOLD response in the participants with obesity (**Contrast 4: Sucrose > Water taste**, healthy weight -0.336 ±0.316 vs. obesity group 0.083 ±0.383, p=0.013). Similarly, a similar difference was also seen between the two groups for the contrast comparing the taste of the chocolate milkshake to water (**Contrast 5: Chocolate milkshake > Water taste**; healthy weight -0.292 ±0.382 vs. obesity group 0.205 ±0.558, p=0.033). In the fed state, no differences were seen between the two groups.

**Anterior insula:** No significant differences in BOLD signal were detected between the fasted and fed state in participants with obesity nor in participants with healthy weight for any of the contrasts. When comparing responses between the two groups in the fasted state, a significantly higher BOLD signal was detected in people with obesity for the contrast between tasting the sucrose solution and water, compared to participants with healthy weight (0.274 ±0.686 vs. -0.030 ±0.512, p=0.049). In the fed state, significant differences between the two groups were seen in the contrasts comparing the taste of sucrose to the taste of water and the taste of the chocolate milkshake to the taste of water, with higher BOLD responses in participants with obesity compared to participants with healthy weight (**Contrast 4: Sucrose > Water taste**; healthy weight -0.436 ±0.325 vs obesity group 0.125 ±0.574, p=0.018) (**Contrast 5: Chocolate milkshake > Water taste**; healthy weight -0.417 ±0.509 vs obesity group 0.154 ±0.506, p=0.015).

**Posterior Insula:** No significant differences in BOLD signal were detected between the fasted and fed state in participants with obesity nor in participants with healthy weight.
for any of the contrasts. Similarly, no differences were seen in between group comparisons in the fasted state for any of the contrasts. In the fed state, a significant difference was noted between participants with healthy weight and participants with obesity when for the contrast comparing the taste of the chocolate milkshake to the taste of water (healthy weight -0.254 ±0.557 vs 0.149 ±0.318; p=0.029).

**Lateral Orbitofronal Cortex:** No significant differences in BOLD signal were detected between the fasted and fed state in participants with obesity nor in participants with healthy weight for any of the contrasts. When comparing between participants with obesity compared to participants with healthy weight significant differences were noted in the fasted state in the contrast comparing BOLD response to sucrose compared to no taste, with higher responses seen in participant with obesity (**Contrast 2: Sucrose > No taste**, healthy weight 0.549 ±0.368 vs. obesity group 1.19 ±0.904, p=0.020). A significant difference was also noted in the contrast comparing response to sucrose to water in the fasted state (**Contrast 4: Sucrose > Water taste**; healthy weight -0.407 ±0.253 vs obesity group -0.031 ±0.641; p=0.047). A significant difference between the two groups was also seen for the same contrast in the fed state (**Contrast 4: Sucrose > Water taste**; healthy weight -0.705 ±0.578 vs. obesity group -0.081 ±0.743; p=0.035). No other significant differences were seen between the two groups in the fed state

**Hypothalamus, Amygdala, Medial Orbitofrontal Cortex:** No significant differences were identified in BOLD signal in the amygdala in participants with obesity nor in participants with healthy weight for any of the contrast in the fasted or the fed state. No significant differences between the two groups were identified.

**5.3.4 Discussion**

The presented data from the TASTER study enabled comparison between the BOLD response in CNS regions relevant to taste perception in people with obesity compared to people with a healthy weight. The taste stimuli used were mineral water, as a neutral taste stimulus without energy content, a sucrose solution as a sweet taste stimulus and a chocolate milkshake, as a sweet and fatty taste stimulus. Studying participants in both
the fed and fasted conditions in a randomised cross-over design enabled comparison of the effect of nutritional status on responses in the CNS appetitive network in people with healthy weight and obesity.

In contrast to the hypothesis, no significant differences were noted in any of the contrasts in participants of healthy weight. This may be a manifestation of low power as the group of healthy weight participants was smaller than intended at the time the study was planned, but could also be a related to the fact that only participants who rated the taste stimuli used in the study as palatable were included in the study.

Tasting water, glucose and the chocolate milkshake all resulted in strong activation of CNS regions relating to taste perception. Water is known to activate the gustatory cortex[28]. No differences in water between groups and conditions suggest this is a neutral taste which does not have an increased reward related value in people with obesity compared to people with healthy weight.

When compared to no taste, whole brain analyses for both the sucrose solution and the chocolate milkshake revealed a similar cluster of increased BOLD activation in in the left dorsal lateral prefrontal cortex. The dorsolateral prefrontal cortex has been linked to food-related decision making, particularly with regard to the cognitive control over food cravings in the context of hedonic eating[29]. A number of fMRI studies have shown increased BOLD activity when instructing participants to suppress food cravings, suggesting an important role in the downregulation of the reward value of food[29-31]. BOLD activity in the dorsal lateral prefrontal cortex has also been shown to correlate with adherence to diet plans in people with obesity[32]. In light of these facts, our findings may suggest that the taste of energy-dense foods directly results in activation of processes aiming to restrict further energy intake. Interestingly, these differences were not affected by the fasted or fed condition, suggesting that this is not a response related to acute changes in energy availability.

The contrast between the taste of the sucrose solution and the taste of water, as well as the taste of the chocolate milkshake compared to water, revealed a cluster
indicating a difference between participants with obesity compared to participants with healthy weight, encompassing parts of the anterior insula and frontal operculum. The anterior insula contains the primary gustatory cortex, encoding information about the primary taste attributes, but also textural and temperature properties of food[33]. In addition, the activity anterior insula has also been implicated in both food-related and non-food related reward[34]. Visual food cues have also been shown to elicit higher responses in the anterior insula in people with obesity compared to healthy weight individuals[34]. An fMRI study in adolescents with obesity has previously shown associations between BOLD activity in the anterior insula and food intake[35]. However, in a recent study by Han et al., CNS responses to the smell of chocolate versus the smell of cucumber were compared in people with obesity compared to healthy weight adults[36]. In this study, chocolate compared to cucumber odour led to increased activation in the insula and frontal operculum[36]. The frontal operculum is involved in processing the flavour of food, as well as the appraisal of the properties of food[37,38]. Together these findings suggest that people with obesity exhibit greater responsivity to the taste of food.

The contrast between milkshake and water also revealed a cluster in participants with obesity compared to healthy weight participants in the left motor cortex. Activation of the motor cortex has previously been seen in a study of healthy weight adolescents while tasting a milkshake compare to a tasteless cue[39]. Although the significance of this cluster of activity is unclear, this may represent increased movement within the oral cavity or indeed an intent for movement with the intention of food acquisition. Furthermore, in the contrast comparing the chocolate milkshake taste to the taste of water, a significant cluster was also identified in people with obesity in the fasted versus the fed state in a region of the anterior cingulate cortex. The anterior cingulate cortex represents the reward value of taste and activity has been correlated with the subjective pleasantness of taste stimuli[40,41]. Additionally, increased anterior cingulate cortex activity has also been seen in people with obesity compared to healthy weight individuals in response to taste stimuli in an fMRI study[42]. Our finding suggests that the fasted state increases the reward value of energy-dense foods in people with obesity.
ROI analysis were carried out for each of the contrasts and results were initially compared in people with healthy weight in the fasted vs the fed state, in people with obesity in the fasted versus the fed state. Subsequently results from the same contrasts were compared in participants with obesity versus participants with healthy weight in the fasted and fed state respectively. No differences were seen in the hypothalamus, amygdala or medial orbitofrontal cortex. A higher response to water compared to no taste was seen in participants with healthy weight in the fed compared to the fasted state. No differences were seen in any of the other contrast relating to the other taste stimuli, suggesting this is not related to energy content of food. One plausible explanation is that this may be a manifestation of thirst[43].

Differences in the fasted state were noted between participants with obesity and participants of healthy weight in the nucleus accumbens and the anterior insula, in the contrasts between sucrose and water as well as chocolate milkshake and water. The nucleus accumbens is involved in taste-related memory formation, food-related decision-making, gustatory-mediated dopamine release and reward responses[44]. These findings suggest a higher taste response, an increased reward value of these tastes compared to water but also an increased motivational response in people with obesity compared to normal weight individuals in response to tasting sucrose and the chocolate milkshake. In the posterior insula however, higher BOLD signal was observed in the contrast between milkshake and water in participants with obesity compared to healthy weight participants in the fed state. The posterior insula has been implicated in encoding sensory information[45]. Interestingly a posterior insular infarct has been shown to result in taste hypersensitivity[46]. Although the exact functions of the posterior insula in taste perception remain incompletely understood, the increased activity in response to tasting the chocolate milkshake may be related to a postprandial decline in taste sensitivity to fat, as discussed in Chapter 4.

The lateral orbitofrontal cortex has been associated with food related decision-making and has also been linked to the reward responses to sweet taste[41,47]. Our findings show higher responses to sucrose compared to no taste and compared to water in
participants with obesity compared to healthy weight participants in the fasted state. Higher activation of the lateral orbitofrontal cortex was also seen in the contrast comparing the response between tasting sucrose and water in participants with obesity in the fed state, suggesting ongoing higher activity in response to sweet taste independent of nutritional status.

5.4 Conclusion
The results of the TASTER study available to date, suggest increased responsiveness both to sweet and to fat-tasting stimuli, as well a higher reward-responses in people with obesity compared to people of healthy weight. Furthermore, these results include novel findings of how taste responses are altered by the fed and fasted state in people with obesity. These findings contribute to the literature on altered taste responses in people with obesity and identify CNS regions involved in the altered eating behaviour observed in people with obesity.

5.5 Future work
Future work on the currently available data from the TASTER study will involve gut hormone profiling and correlating the results with the activity observed in response to the different tastes both in the fasted and the fed state. Evaluating how both dietary, pharmacological and surgical treatments for obesity impact upon these responses will be essential in order to improve treatment strategies, aiming at addressing the increased reward responses to the taste of food in people with obesity.
5.5 Chapter 5 References


6. Chapter 6: Adipocytokines relevant to COVID-19 in people with severe obesity and following bariatric surgery

6.1 Introduction

The COVID-19 pandemic has highlighted the need to improve our understanding of the links between the physiological consequences of obesity and the increased risk of severe illness from infection. This is relevant both given the potential of further waves of the current pandemic, but also in the context of future outbreaks of viral infections. Identifying people at the highest risk of developing critical illness from infection will allow targeted protective measures in the context of imminent exposure but will also allow to prioritise higher risk patients for treatment. TNFa, IL-6 and GDF15 have all been associated with an increased risk of critical illness in COVID-19[1-3]. VAT and immune cell accumulation in VAT are likely the source of the majority of supraphysiological secretion of these cytokines in people with obesity[4,5]. VAT itself has also been associated with an increased risk of requiring ICU level care in people with obesity and COVID-19[6]. Data from CT scans performed on admission to hospital in patients with COVID-19 have highlighted the association between VAT and critical illness in COVID-19[7,8].

During the cytokine storm caused by COVID-19, significant elevations of IL-10 have also been noted[9]. Although this was initially thought to be a negative feedback mechanism to suppress inflammation, several studies now suggest that a pro-inflammatory IL-10 elevation may play a pathological role in COVID-19 severity[9]. One proposed mechanism is that IL-10 in severe COVID-19 contributes to T cell exhaustion, theoretically via overactivation and proliferation[10]. Furthermore, adiponectin is known to have a protective effect on lung capillaries and low adiponectin levels have been linked to respiratory failure secondary to COVID-19 pneumonia[11]. Taken together, these data suggest a specific role for adiposity-mediated cytokines in the pathogenesis of severe COVID-19.
In addition to cytokines, population-wide studies have also suggested that metabolomic profiling, which is able to identify biomarkers for T2DM and cardiovascular disease, may also be able to identify susceptibility to critical illness from infectious diseases [12,13]. For instance, the pro-inflammatory metabolic biomarker glycoprotein acetyl (GlycA), a marker of oxidative stress, which has previously been shown to predict cardiovascular disease, has been linked to an increased risk of severe disease from infection, particularly in the context of sepsis and pneumonia[12]. Furthermore, direct links between circulating fatty acids and obesity-related inflammation have been proposed[14]. Long-chain saturated fatty acids (SFA) in particular, have been shown to directly trigger pro-inflammatory responses, by promoting the secretion of cytokines such as TNFa and IL-6[15]. Additionally, branched-chain amino acids (BCAA), which include leucine, isoleucine and valine, have been associated with obesity, chronic inflammation and T2DM[16]. Importantly, a growing body of evidence suggests that BCAA levels are a direct marker of VAT, which may have significant implications for their potential use as a diagnostic and prognostic marker in people with obesity[17,18].

Julkunen et al., recently published a multi-biomarker score, encompassing results of 25 biomarkers obtained from metabolomic profiling, including fatty acids, amino acids, and lipids, and demonstrated this associated both with an enhanced susceptibility to severe COVID-19 and an increased risk of severe pneumonia[19]. In this study, samples from a UK-based biobank, collected 10 years prior to the COVID-19 pandemic from healthy individuals, were used to investigate if these biomarkers associate with an increased risk of severe infection in a UK general population settings[19]. Their multi-biomarker score, developed originally to assess the risk of severe pneumonia, was assessed as potential predictor of severe COVID-19 on a sample of approximately 100 000 people with COVID-19 outcome data, using the severe pneumonia data as a control and demonstrated that the score identifies an increased risk of severe illness from COVID-19[19].

The degree to which obesity-related chronic inflammation can be reversed and the respective effectiveness of currently available treatments in improving inflammation
and mitigating the associated risks remain poorly understood. A series of studies have shown improved cytokine profiles following dietary weight loss as well as bariatric surgery, with reductions in circulating TNFa and IL-6 levels observed in a number of these studies[20-24]. Findings regarding the improvement of these cytokine profiles however remain controversial, with other studies showing no change following weight loss[25,26]. Methodological differences as well as variability in the metabolic characteristics of study populations between these cohorts are likely to underlie these differences and highlight the need for studies investigating both the effects of obesity and weight loss on circulating cytokines in the context of obesity-associated chronic inflammation. In addition, it also still remains unclear whether the benefits of weight loss on these cytokine profiles pertain only to individuals with a pre-existing proinflammatory state.

To date, there are no diagnostic or prognostic tools available to characterise the chronic inflammatory state in obesity and how this relates to the risk of developing critical illness in the context of infection. Effective preventative measures for both T2DM and cardiovascular disease, as well as for mitigating the impact of infection, will require accurate prognostic tools to identify those at risk. Therefore, the work presented in this chapter aims to provide preliminary and exploratory data on adipocytokines and biomarkers of chronic inflammation which have been linked to an increased risk of poor outcomes from COVID-19 and how these are altered by bariatric surgery.

The aims of this study were to:

1. Investigate the effect of bariatric surgery on circulating levels of adipocytokines linked to COVID-19 prognosis (adiponectin, TNFa, IL-6, IL-10 and GDF15) in people with and without T2DM.
2. Investigate the effects of bariatric surgery on circulating biomarkers linked to an increased risk of metabolic disease and severe illness from COVID-19.
6.2 Methods

6.2.1 Recruitment and setting
Participants were recruited from the UCLH Bariatric Centre for Weight Management and Metabolic Surgery. Participation in the study was voluntary and informed written consent was obtained. The study received National Health Service Research Ethics Committee approval (ID#09/H0715/65) and was undertaken in accordance with the Helsinki Declaration.

6.2.2 Participants
Participants were recruited from the UCLH Bariatric Centre for Weight Management and Metabolic Surgery. Participation in the study was voluntary and informed written consent was obtained. The study had National Health Service Research Ethics Committee approval (ID#09/H0715/65) and was undertaken in accordance with the Helsinki Declaration.

A cohort of adults with severe obesity, defined by a BMI ≥40kg/m² or ≥35kg/m² in the presence of obesity-associated co-morbidities, who were due to undergo a primary RYGB or SG were invited to participate. The study’s exclusion criteria were HIV and hepatitis B, pregnancy and lactation and any acute or chronic conditions likely to impact upon post-operative weight loss. Participants with and without T2DM undergoing a RYGB and SG respectively, were recruited into separate groups, matched for age, sex and post-operative weight loss. In participants with T2DM, people with a disease duration of T2DM greater than 10 years and those on insulin therapy were excluded. Procedure selection between RYGB and SG was based on a multidisciplinary team assessment, taking into account individual circumstances and personal preference and fulfilled the criteria outlined by the NICE guidelines[27].

6.2.3 Study visits
Study visits took place at the UCLH Clinical Research Facility. Participants attended for their baseline visit (pre-surgery) within two weeks from their date of surgery and for their follow up visits at 6 weeks, 6 months and 12 months post-surgery. During the pre-
operative period, patients are requested to follow a soft-textured 1000kcal diet, for the purpose of reducing liver size prior to surgery. In order to standardise the pre-study conditions, participants were asked to follow the same diet for 24h prior to their subsequent study visits.

At each visit, participants’ medical and drug histories were reviewed and recorded. Weight was recorded using validated weighing scales, height was measured using a wall-mounted stadiometer and BMI was calculated. At post-operative study visits, percentage weight loss (%WL) was calculated using the following formula: %WL = ([baseline body weight – follow up visit body weight]/ baseline body weight) x 100.

6.2.4 Sample collection and processing
Samples for adipocytokine analyses were drawn into EDTA tubes primed with aprotinin, a kallikrein inhibitor (aprotinin 10,000KIU/ml, Nordic Pharma, 5000 units per mL of blood). Blood samples for metabolomics were drawn into EDTA tubes. Collection tubes were immediately transferred into an ice box and centrifuged at 1800rpm at 4°C for 10 minutes following collection. Plasma was separated and stored in aliquots at -80°C until analysis.

6.2.5 Cytokine assays
For all assays, samples from each participant were analysed on the same plate and assay kits with same LOT number were used where possible. Samples were measured in duplicates and the mean of the duplicates was used as a raw data value for data reduction. Where significant discrepancies existed between duplicates the sample was re-measured on a new assay.

6.2.5.1 Adiponectin
Adiponectin was measured using a human adiponectin ELISA assay (Millipore; sensitivity 0.2ng/mL; inter-assay variability 7.4% at 17.7ng/mL, 0.9% at 29.1ng/mL; intra-assay variability 8.4% at 17.7ng/mL, 2.4% at 29.1ng/mL). A 500-fold dilution of plasma samples was performed prior to analysis.
6.2.5.2 TNF-a

TNF-a was measured using a human TNFα Quantikine ELISA assay (R&D systems; sensitivity 2.1-6.23pg/mL; intra-assay variability 2.6% at 155pg/mL, inter-assay variability 7.3% at 155pg/mL).

6.2.5.3 IL-6

IL-6 was measured using a human IL-6 Quantikine ELISA assay (R&D systems; sensitivity 0.70pg/mL, inter-assay variability 4.4% at 15.8pg/mL, 3.1% at 95.6pg/mL; intra-assay variability 3.7% at 16.4pg/mL, 2.5% at 98.8pg/mL).

6.2.5.4 IL-10

IL-10 was measured using a human IL-10 Quantikine ELISA assay (R&D systems; sensitivity <3.9pg/mL, inter-assay variability 6.6% at 24.4pg/mL, intra-assay variability 7.6% at 23.6pg/mL).

6.2.5.5 GDF15

GDF15 was measured using a human GDF15 Quantikine ELISA assay (R&D systems; sensitivity 0.0-0.4pg/mL; intra-assay variability 1.8% at 238pg/mL, 2.2% at 456pg/mL and 2.8% at 886pg/mL; inter-assay variability 6.0% at 225pg/mL, 4.7% at 442pg/mL and 5.6% at 900pg/mL). A 4-fold dilution of plasma samples was performed prior to analysis.

6.2.6 Metabolomic biomarkers

Metabolomic samples were analysed by Nightingale Health (Nightingale Health Plc; Helsinki, Finland). Biomarkers were measured using high-throughput nuclear magnetic resonance (NMR) spectroscopy (Nightingale Health Plc; biomarker quantification version 2020). This method provides simultaneous quantification of 249 metabolic biomarker, including routine lipids, lipoprotein subclass profiling with lipid concentrations within 14 subclasses, fatty acid composition, and various low-molecular-weight metabolites such as amino acids, ketone bodies, and glycolysis.
metabolites quantified in molar concentration units. The metabolic biomarkers measured using the Nightingale Health platform have been used in numerous published epidemiological studies (https://nightingalehealth.com/publications).

6.2.7 Infectious disease score

A score, based on the biomarkers identified in the ‘infectious disease score’ by Julkunen et al. [19], was calculated for each participant. In order to enable comparison of how the total score was altered by bariatric surgery, the absolute value recorded for each biomarker was multiplied by the respective weighting coefficient (β-weight, Table 6.1). The score subsequently calculated as β1X1 + β2X2 + ... + β25X25, with Xi denoting the measured concentration for the ith biomarker and βi denoting the weighting coefficient.
Table 6.1: Biomarkers and weights for each biomarker included in the multi-biomarker infectious disease score devised by Julkunen et al.[19].

<table>
<thead>
<tr>
<th>Biomarker</th>
<th>β-weight for 'Infectious disease score'</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glycoprotein acetyls (GlycA)</td>
<td>0.3713</td>
</tr>
<tr>
<td>DHA %</td>
<td>0.2533</td>
</tr>
<tr>
<td>Creatinine</td>
<td>0.2170</td>
</tr>
<tr>
<td>MUFA</td>
<td>0.1693</td>
</tr>
<tr>
<td>Apolipoprotein B / Apolipoprotein A1</td>
<td>0.1388</td>
</tr>
<tr>
<td>Tyrosine</td>
<td>0.1375</td>
</tr>
<tr>
<td>Isoleucine</td>
<td>0.1091</td>
</tr>
<tr>
<td>SFA %</td>
<td>0.0965</td>
</tr>
<tr>
<td>Glucose</td>
<td>0.0928</td>
</tr>
<tr>
<td>Lactate</td>
<td>0.0772</td>
</tr>
<tr>
<td>Omega-6/Omega-3</td>
<td>0.0642</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>0.0289</td>
</tr>
<tr>
<td>Total cholesterol</td>
<td>-0.0116</td>
</tr>
<tr>
<td>Omega-6 %</td>
<td>-0.0493</td>
</tr>
<tr>
<td>Alanine</td>
<td>-0.0498</td>
</tr>
<tr>
<td>PUFA</td>
<td>-0.0578</td>
</tr>
<tr>
<td>Glycine</td>
<td>-0.0648</td>
</tr>
<tr>
<td>Histidine</td>
<td>-0.0987</td>
</tr>
<tr>
<td>PUFA %</td>
<td>-0.1404</td>
</tr>
<tr>
<td>Valine</td>
<td>-0.1812</td>
</tr>
<tr>
<td>Leucine</td>
<td>-0.1844</td>
</tr>
<tr>
<td>Albumin</td>
<td>-0.1914</td>
</tr>
<tr>
<td>Omega-3</td>
<td>-0.2208</td>
</tr>
<tr>
<td>LDL cholesterol</td>
<td>-0.2466</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>-0.2652</td>
</tr>
</tbody>
</table>

The table indicates the weights for the 25 biomarkers that form part of the multi-biomarker infectious disease score, devised by Julkunen et al.[19]. DHA: docosahexaenoic acid; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids; SFA: saturated fatty acids.
6.2.8 Statistical analysis
Statistical analysis was conducted using GraphPad Prism Version 8 and SPSS Version 25 (IBM Statistics). The Gaussian distribution was assessed by D’Agostino-Pearson normality test and parametric (t-tests) or non-parametric tests were used as appropriate. Change from baseline was calculated by subtracting the baseline value from each value recorded at the follow-up visits after bariatric surgery. Change from baseline in figures is plotted as a mean and SEM. One-way ANOVA repeated measures models were used to analyze repeat measurements over time. A correction for multiple comparisons was applied using a Bonferroni correction. When data were not normally distributed a non-parametric ANOVA Friedman’s test was performed, with a Dunn’s correction for multiple comparisons. Pearson’s correlation analyses were performed to investigate the relationship between recorded cytokines/biomarkers and parameters of body composition. Results are expressed as calculated means with standard deviation (SD). Interquartile ranges were calculated by identifying the 1st and 3rd quartile for each set of results respectively. Statistical significance was assumed at the 0.05 level.

6.2.9 Sample size estimation
Sample size was estimated based on some pilot data available at the UCL Centre for Obesity Research from metabolomic biomarker analysis in a cohort of research participants who underwent bariatric surgery. Based on the observed change in a number of relevant biomarkers in this pilot sample, it was estimated that a sample size of 12 participants would be required in each group of participants with and without T2DM respectively, for 80% power to detect meaningful changes following bariatric surgery in metabolomic biomarkers. The results in inflammatory cytokines were treated as exploratory.

6.3 Results

6.3.1 Participant demographics
Demographic data and participant characteristics prior to bariatric surgery are summarised in Table 6.2. In line with clinical recommendations prior to bariatric
surgery, all the participants in this study were non-smokers. In terms of ethnicity, in the group without T2DM, 50.0% (n=11) of participants were white, 31.8% (n=7) of Black/African/Caribbean origin, 4.5% (n=1) of Asian/Asian British/Asian Indian origin, 9.1% (n=2) of other ethnicity and 4.5% (n=1) did not disclose their ethnicity. In the group of participants with T2DM, 64.3% (n=9) of participants were white, 7.1% (n=1) Black/African/Caribbean origin, 7.1% (n=1) of Asian/Asian British/Asian Indian origin and 21.4% (n=3) did not disclose their ethnicity.
Table 6.2: Participant characteristics for participants with and without T2DM

<table>
<thead>
<tr>
<th></th>
<th>Age (years)</th>
<th>Sex</th>
<th>BMI at surgery (kg/m²)</th>
<th>12 month %WL (%)</th>
<th>Procedure</th>
</tr>
</thead>
<tbody>
<tr>
<td>Participants without T2DM (n=22)</td>
<td>49.6 ±8.0</td>
<td>100% Female</td>
<td>44.4 ±4.4</td>
<td>27.3 ±5.7</td>
<td>50% RYGB, 50% SG</td>
</tr>
<tr>
<td>Participants with T2DM (n=14)</td>
<td>54.3 ±8.5</td>
<td>100% Female</td>
<td>44.2 ±5.7</td>
<td>24.8 ±6.8</td>
<td>50% RYGB, 50% SG</td>
</tr>
<tr>
<td>p value</td>
<td>p=0.104</td>
<td>p=1.000</td>
<td>p=0.861</td>
<td>p=0.260</td>
<td>p=1.000</td>
</tr>
</tbody>
</table>

T2DM: Type 2 Diabetes Mellitus, BMI: Body Mass Index, RYGB: Roux-en-Y Gastric Bypass, SG: Sleeve Gastrectomy, %WL: Percentage Weight Loss

In the group of participants with T2DM, the mean duration of T2DM from diagnosis was 2.9 years ±2.3 and the mean HbA1c at baseline was 6.6% ±0.6. At baseline, 57.1% (n=8) were on treatment with oral glucose-lowering medication, all of whom were taking metformin. Of these, two participants were on combined oral therapy with a second agent, gliclazide in one case and canagliflozin in the other. At 12 months of follow-up, mean HbA1c was 5.6% ±0.4 and 100% of participants achieved remission of T2DM, all of which discontinued their glucose-lowering medication.

6.3.2 Adipocytokine results

6.3.2.1 Baseline values
Table 6.3 summarises the baseline values measured for participants with and without T2DM respectively for circulating adiponectin, TNFa, IL-6, IL-10, GDF15. There were no significant differences between the cohorts with and without T2DM in circulating levels of adiponectin, TNFa, IL-6, IL-10, however GDF15 was significantly higher in participants with T2DM (563.3pg/mL ±200.9 vs. 1108.0pg/mL ±498.7, p<0.001).
Table 6.3: Circulating levels of adipocytokines measured at baseline in participants with and without T2DM

<table>
<thead>
<tr>
<th>Adipocytokine</th>
<th>Participants without T2DM (n=22)</th>
<th>Participants with T2DM (n=14)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adiponectin (ng/mL)</td>
<td>8044 ±3239</td>
<td>7014 ±2328</td>
<td>0.310</td>
</tr>
<tr>
<td>TNFa (pg/mL)</td>
<td>12.8 ±7.2</td>
<td>8.8 ±4.0</td>
<td>0.097</td>
</tr>
<tr>
<td>IL-6 (pg/mL)</td>
<td>9.3 ±26.5</td>
<td>6.6 ±6.0</td>
<td>0.465</td>
</tr>
<tr>
<td>IL-10 (pg/mL)</td>
<td>9.2 ±8.0</td>
<td>7.7 ±5.2</td>
<td>0.558</td>
</tr>
<tr>
<td>GDF15 (pg/mL)</td>
<td>563.3 ±200.9</td>
<td>1108.0 ±498.7</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

T2DM: Type 2 Diabetes Mellitus, TNFa: Tumour Necrosis Factor alpha, IL-6: Interleukin 6, IL-10: Interleukin 10, GDF15: Growth Differentiation Factor 15.

6.3.2.2 Circulating adipocytokines following bariatric surgery

6.3.2.2.1 Adiponectin

Circulating levels of adiponectin rose following bariatric surgery both in people with and without T2DM (Figure 6.1A). In participants without T2DM adiponectin levels were 8044ng/mL ±3239 at baseline, 8448ng/mL ±3901 at 6 weeks, 9018ng/mL ±3596 at 6 months and 11841 ±5648 at 12 months from surgery. This represents a significant elevation from baseline (p=0.001). Repeated measures ANOVA identified significant elevations from baseline to 12 months (mean difference -3797, CI -6880 to -714.8, p= 0.010), from 6 weeks to 12 months (mean difference -3394, CI -6378 to -410, p= 0.020) and from 6 months to 12 months (mean difference -2823, CI -5123 to -520, p= 0.010).

In participants with T2DM a significant reduction in circulating adiponectin levels was also seen following bariatric surgery (ANOVA repeated measures p=0.005). Mean circulating adiponectin levels at baseline were 7014ng/mL ±2328, at 6 weeks post-surgery 8630ng/mL ±3619, at 6 months 9653ng/mL ±4647 and at 12 months
12178ng/mL ±5925. A significant reduction was identified from baseline to 12 months from surgery (mean difference -5164, CI -9393 to -934, p=0.013), reflecting a similar pattern of adiponectin secretion following bariatric surgery in people with and without T2DM.

Figure 6.1: Circulating adiponectin levels before and after bariatric surgery in people with and without T2DM

Figure illustrating adiponectin levels before and after bariatric surgery. A: Plasma adiponectin values recorded at baseline, 6 weeks, 6 months and 12 months following bariatric surgery in people without T2DM (error bars: SD). Repeated measures ANOVA identified significant elevations from baseline to 12 months (mean difference -3797, CI -6880 to -714.8, p= 0.010), from 6 weeks to 12 months (mean difference -3394, CI -6378 to -410, p= 0.020) and from 6 months to 12 months (mean difference -2823, CI -5123 to -520, p= 0.010)

B: Plasma adiponectin values recorded at baseline, 6 weeks, 6 months and 12 months following bariatric surgery in people with T2DM (error bars: SD). Mean circulating adiponectin levels at baseline were 7014ng/mL ±2328, at 6 weeks post-surgery 8630ng/mL ±3619, at 6 months 9653ng/mL ±4647 and at 12 months 12178ng/mL ±5925. A significant reduction was identified from baseline to 12 months from surgery (mean difference -5164, CI -9393 to -934, p=0.013).

C: Change from baseline in plasma adiponectin values recorded 6 weeks, 6 months and 12 months following bariatric surgery in people without T2DM. Filled squares indicate mean change in adiponectin.
D: Change from baseline in plasma adiponectin values recorded 6 weeks, 6 months and 12 months following bariatric surgery in people with T2DM. Open circles squares indicate mean change in adiponectin. (* indicates p<0.05). T2DM: Type 2 Diabetes Mellitus.

6.3.2.2.2 TNFa

In participants without T2DM, plasma TNFa levels were measured at 12.8pg/mL ±7.2 at baseline, 14.6pg/mL ±7.7 at 6 weeks, 12.9pg/mL ±10.2 at 6 months and 12.9pg/mL ±10.9 at 12 months from surgery. No statistically significant change from baseline was recorded (p=0.666) (Figure 6.2A).

In participants with T2DM, mean circulating TNFa levels were 8.9pg/mL ±4.0 at baseline, 11.4pg/mL ±6.5 at 6 weeks, 12.4pg/mL ±9.9 at 6 months and 12.1pg/mL ±10.3 at 12 months following bariatric surgery. Repeated measures ANOVA highlighted no significant differences (p=0.449) (Figure 6.2B).

It is however notable, that a large variability both in terms of baseline TNFa samples but also in terms of their change following surgery was noted within the cohort of participants. Out of participants without T2DM, a reduction in TNFa from baseline to 12 months was seen in 50% (n=11) of participants and an elevation from baseline to 12 months in 50%. Participants whose TNFa levels rose following bariatric surgery had a baseline mean TNFa level of 16.4pg/mL ±8.2, whereas those whose TNFa levels reduced had mean baseline TNFa levels of 9.2 ±3.3, p=0.015. There were no significant differences in their age, baseline BMI or post-operative %WL. In the group of participants with T2DM, TNFa levels dropped following surgery in 9 participants (64.3%) and rose in 5 participants (35.7%). Although TNFa levels were higher at baseline in participants whose levels dropped, compared to those in whom it did not, the difference did not reach statistical significance (7.3pg/mL ±3.8, vs. 9.8 ±4.1, p=0.381).
**Figure 6.2: TNFa levels following bariatric surgery in people with and without T2DM**

![Graph A: Participants without T2DM](image1)

![Graph B: Participants with T2DM](image2)

Figure illustrating TNFa levels before and after bariatric surgery. A: Plasma TNFa values recorded at baseline, 6 weeks, 6 months and 12 months following bariatric surgery in people without T2DM (error bars: SD). TNFa levels were measured at 12.8pg/mL ± 7.2 at baseline, 14.6pg/mL ± 7.7 at 6 weeks, 12.9pg/mL ± 10.2 at 6 months and 12.9pg/mL ± 10.9 at 12 months from surgery. No statistically significant change from baseline was recorded (p=0.666) B: Plasma TNFa values recorded at baseline, 6 weeks, 6 months and 12 months following bariatric surgery in people with T2DM (error bars: SD). TNFa levels were 8.9pg/mL ± 4.0 at baseline, 11.4pg/mL ± 6.5 at 6 weeks, 12.4pg/mL ± 9.9 at 6 months and 12.1pg/mL ± 10.3 at 12 months (p=0.449). TNFa: Tumour Necrosis Factor alpha, T2DM: Type 2 Diabetes Mellitus.

### 6.3.2.2.3 IL-6

Mean circulating IL-6 levels at baseline in participants without T2DM were 9.3pg/mL ± 26.5. IL-6 levels dropped following bariatric surgery to 7.9pg/mL ± 17.4 at 6 weeks, to 7.1pg/mL ± 15.6 at 6 months and to 5.8pg/mL ± 16.7 at 12 months following bariatric surgery (ANOVA p=0.002). Significant reductions in IL-6 were noted from baseline to 12 months (9.3pg/mL ± 26.5 to 5.8pg/mL ± 16.7, p=0.012), from 6 weeks to 12 months (7.9pg/mL ± 17.4 to 5.8pg/mL ± 16.7, p=0.012) and from 6 months to 12 months (7.1pg/mL ± 15.6 to 5.8pg/mL ± 16.7, p=0.001) (Figure 6.3).

In participants with T2DM, circulating IL-6 levels were 4.6pg/mL ± 6.0 at baseline, 3.8pg/mL ± 1.8 at 6 weeks post-surgery, 1.9pg/mL ± 1.2 at 6 months and 3.5 ± 5.3 at 12 months from surgery (ANOVA p=0.008). A significant reduction was recorded from baseline to 6 months (4.6pg/mL ± 6.0 to 1.9pg/mL ± 1.2, p=0.04) and from 6 weeks to 6 months (3.8pg/mL ± 1.8 to 1.9pg/mL ± 1.2, p=0.016).
Variabilities in IL-6 secretion following surgery were noted. Levels dropped from baseline to 12 months in 77.3% (n=17) and rose in 22.7% (n=5). IL-6 levels were higher in people with a post-operative drop compared to those with a rise in IL-6 but this did not reach statistical significance (11.4pg/mL ±30.0 vs. 2.4pg/mL ±1.8, p=0.068). In participants with T2DM baseline IL-6 levels in people with a post-operative drop (64.3%, n=9) were 5.7pg/mL ±7.4 and in those with a post-operative rise (35.7%, n=5) 2.6pg/mL ±0.8 (p=0.156).

Figure 6.3: IL-6 levels before and after bariatric surgery in people with and without T2DM

Figure illustrating IL-6 levels before and after bariatric surgery. A: Plasma IL-6 values recorded at baseline, 6 weeks, 6 months and 12 months following bariatric surgery in people without T2DM (error bars: SD). Baseline IL-6 was 9.3pg/mL ±26.5. IL-6 levels dropped to 7.9pg/mL ±17.4 at 6 weeks, to 7.1pg/mL ±15.6 at 6 months and to 5.8pg/mL ±16.7 at 12 months following bariatric surgery (p=0.002). Significant reductions in IL-6 were noted from baseline to 12 months (9.3pg/mL ±26.5 to 5.8pg/mL ±16.7, p=-0.012), from 6 weeks to 12 months (7.9pg/mL ±17.4 to 5.8pg/mL ±16.7, p=-0.012) and from 6 months to 12 months (7.1pg/mL ±15.6 to 5.8pg/mL ±16.7, p=-0.001). B: Plasma IL-6 values recorded at baseline, 6 weeks, 6 months and 12 months following bariatric surgery in people with T2DM (error bars: SD). In participants with T2DM, circulating IL-6 levels were 4.6pg/mL ±6.0 at baseline, 3.8pg/mL ±1.8 at 6 weeks post-surgery, 1.9pg/mL ±1.2 at 6 months and 3.5 ±5.3 at 12 months from surgery (ANOVA p=0.008). A
significant reduction was recorded from baseline to 6 months (4.6pg/mL ±6.0 to 1.9pg/mL ±1.2, p=0.04) and from 6 weeks to 6 months (3.8pg/mL ±1.8 to 1.9pg/mL ±1.2, p=0.016). C: Change from baseline in plasma IL-6 values recorded 6 weeks, 6 months and 12 months following bariatric surgery in people without T2DM. Filled squares indicate mean change in plasma IL-6. (error bars: SEM). D: Change from baseline in plasma IL-6 values recorded 6 weeks, 6 months and 12 months following bariatric surgery in people with T2DM. Circles indicate mean change in IL-6. (error bars: SEM). (* indicates p<0.05 and ** p<0.01) IL-6: Interleukin 6, T2DM: Type 2 Diabetes Mellitus, SEM: Standard error of the mean.

6.3.2.2.4 IL-10

Following bariatric surgery, IL-10 levels did not significantly change in participants without T2DM. Circulating IL-10 levels were 9.2pg/mL ±7.9 pre-surgery, 9.4pg/mL ±5.1 at 6 weeks, 9.6pg/mL ±5.4 at 6 months and 5.6pg/mL ±5.6 at 12 months from surgery (ANOVA p=0.741). A reduction was seen at 12 months from surgery although this did not reach statistical significance (Figure 6.4).

In participants with T2DM, a significant change in plasma IL-10 levels was seen following bariatric surgery (ANOVA p=0.041). From a baseline of 7.7pg/mL ±5.2, a non-significant increase was seen at 6 weeks to 9.4pg/mL ±4.9. At 6 months circulating IL-10 levels were 7.5pg/mL ±3.4 and at 12 months from surgery 6.7pg/mL ±3.6. The reduction noted from 6 weeks to 12 months was no longer significant after adjusting for multiple comparisons (to 9.4pg/mL ±4.9 to 6.7pg/mL ±3.6, p=0.063).

A similar heterogeneity in the response of IL-10 secretion following bariatric surgery compared to other cytokines was seen. In the cohort without T2DM, IL-10 levels reduced in 54.5% (n=12) and rose in 45.5% (n=10). Baseline IL-10 levels were 12.0 pg/mL ±9.1 in participants with a subsequent drop, compared to 5.1pg/mL ±3.7 in participants with a post-operative rise in IL-10 (p=0.006). In participants with T2DM, IL-10 levels a year from surgery had dropped in 64.3% (n=9) participants and risen in 35.7% (n=5). Mean IL-10 levels at baseline were 9.1pg/mL ±5.9 in those with a post-operative IL-10 reduction and 5.2 pg/mL ±1.6 in those with a post-surgery IL-10 elevation (p=0.184).
Figure 6.4: Circulating IL-10 levels before and after bariatric surgery in people with and without T2DM

A. Participants without T2DM

B. Participants with T2DM

C. Participants without T2DM

D. Participants with T2DM

Figure illustrating IL-10 levels before and after bariatric surgery. A: Plasma IL-10 values recorded at baseline, 6 weeks, 6 months and 12 months following bariatric surgery in people without T2DM (error bars: SD). Circulating IL-10 levels were 9.2pg/mL ±7.9 pre-surgery, 9.4pg/mL ±5.1 at 6 weeks, 9.6pg/mL ±5.4 at 6 months and 5.6pg/mL ±5.6 at 12 months from surgery (p=0.741). B: Plasma IL-10 values recorded at baseline, 6 weeks, 6 months and 12 months following bariatric surgery in people with T2DM (error bars: SD). From a baseline of 7.7pg/mL ±5.2, a non-significant increase was seen at 6 weeks to 9.4pg/mL ±4.9. At 6 months circulating IL-10 levels were 7.5pg/mL ±3.4 and at 12 months from surgery 6.7pg/mL ±3.6. The reduction noted from 6 weeks to 12 months was no longer significant after adjusting for multiple comparisons (to 9.4pg/mL ±4.9 to 6.7pg/mL ±3.6, p=0.063). C: Change from baseline in plasma IL-10 values recorded 6 weeks, 6 months and 12 months following bariatric surgery in people without T2DM. Filled squares indicate change in plasma IL-10. D: Change from baseline in plasma IL-10 values recorded 6 weeks, 6 months and 12 months following bariatric surgery in people with T2DM. Open circles indicate change in IL-10. IL-10: Interleukin 10, T2DM: Type 2 Diabetes Mellitus.

6.3.2.2.5 GDF15

In participants without T2DM, GDF15 levels were 563pg/mL ±200.9 at baseline, 723pg/mL ±306.3 at 6 weeks, 596.6 pg/mL ±186.3 at 6 months and 548.2pg/mL ±170.4 at 12 months from surgery (ANOVA p=0.005). Repeated measures analysis identified a
significant drop from 6 weeks to 12 months post-surgery (mean difference 175.6pg/mL, CI 11.60 to 339.5, p=0.031) (Figure 6.5).

In participants with T2DM a gradual reduction in GDF15 levels was seen following surgery. From a baseline of 1108pg/mL ±498.7 pre-surgery, levels dropped to 1027pg/mL ±538.1 at 6 weeks, 893.5pg/mL ±380.3 at 6 months and 797.3pg/mL ±300.9 at 12 months following bariatric surgery (ANOVA p=0.023). The repeated measures analysis identified a significant drop from pre-surgery to 12 months following bariatric surgery (mean difference 310.4pg/mL, CI 27.1 to 593.6, p=0.032) (Figure 6.5).

Similar to the other cytokines, in the cohort without T2DM, GDF15 levels reduced in 50% (n=11) and rose in 50% (n=11). Baseline plasma GDF levels were higher in participants with a subsequent GDF15 level reduction compared to those with a post-operative rise in GDF15 (679.2 pg/mL ±182.7 vs. 447.3 pg/mL ±147.7, p=0.004). In participants with T2DM, the difference in baseline GDF15 levels between the 69.2% of participants with a post-surgery drop in GDF15 levels compared to the 30.8% with a post-bariatric surgery rise did not reach statistical significance (1213.0pg/mL ±509.5 vs. 869.9pg/mL ±440.0, p=0.297).
Figure 6.5: Circulating GDF15 levels before and after bariatric surgery in people with and without T2DM

A. Participants without T2DM

B. Participants with T2DM

C: Change from baseline in plasma GDF15 values recorded 6 weeks, 6 months and 12 months following bariatric surgery in people without T2DM. Filled squares indicate mean change in GDF15. (Error bars: SEM).

D: Change from baseline in plasma GDF15 values recorded 6 weeks, 6 months and 12 months following bariatric surgery in people with T2DM. Open circles indicate mean change in GDF15. (Error bars: SEM).

GDF15: Growth Differentiation Factor 15, T2DM: Type 2 Diabetes Mellitus, SEM: Standard error of the mean.
6.3.3 *Metabolomic results*

Results from biomarkers relevant to chronic inflammation and potential adverse outcomes in the context of metabolic and infectious disease from the metabolomic profiling panel were selected for longitudinal analysis before bariatric surgery, and at 6 weeks, 6 months and 12 months from surgery. At baseline, there were no differences in circulating levels of GlycA (1.00mmol/L ±0.16 vs. 0.93mmol/L ±0.10, p=0.203) or fatty acids (11.4mmol/L ±2.2 vs. 11.5 mmol/L ±2.4, p=0.858) in participants without T2DM compared to those with T2DM. However, total BCAA levels were lower in participants without T2DM compared to participants with T2DM (0.27mmol/L ± 0.16 vs. 0.41mmol/L ±0.08, p=0.002).

6.3.3.1 *Glycoprotein acetyl (GlycA)*

GlycA levels in participants without T2DM reduced significantly following surgery (p<0.001). GlycA levels were 1.00mmol/L ±0.16 at baseline, 0.88mmol/L ±0.10 at 6 weeks, 0.86 mmol/L±0.11 at 6 months and 0.79mmol/L ±0.09 at 12 months from surgery. Repeated measures analysis showed significant reductions in GlycA from baseline to 6 weeks (mean difference 0.12, CI 0.02 to 0.23, p=0.017), baseline to 6 months weeks (mean difference 0.15, CI 0.05 to 0.25, p=0.002), baseline to 12 months (mean difference 0.21, CI 0.11 to 0.30, p<0.001), and from 6 weeks to 12 months (mean difference 0.08, CI 0.02 to 0.15, p<0.007), as well as from 6 months to 12 months following bariatric surgery (mean difference 0.05, CI 0.01 to 0.11, p=0.015) (Figure 6.6).

In participants with T2DM, GlycA levels were 0.93mmol/L ±0.10 at baseline, 0.88mmol/L ±0.11 at 6 weeks, 0.86 mmol/L±0.10 at 6 months and 0.81mmol/L ±0.12 at 12 months from surgery (ANOVA p<0.001). Repeated measures analysis identified a significant drop from baseline to 12 months surgery (mean difference 0.14, CI 0.06 to 0.23, p=0.001) and from 6 weeks to 12 months surgery (mean difference 0.08, CI 0.02 to 0.14, p=0.008) (Figure 6.6).
6.3.3.2 Fatty acids

Total fatty acids in participants without T2DM were 11.4 mmol/L ± 2.2 prior to bariatric surgery, 10.1 mmol/L ± 1.9 at 6 weeks, 10.7 mmol/L ± 2.1 at 6 months and 10.7 mmol/L ± 1.9 at 12 months from surgery in participants without T2DM (ANOVA p=0.033). A significant reduction was noted from baseline to 6 weeks post-surgery (mean difference 1.2, CI 0.01 to 2.5, p=0.047). In participants with T2DM, total fatty acids were 11.5 mmol/L ± 2.4 at baseline, 10.7 mmol/L ± 1.8 at 6 weeks, 11.1 mmol/L ± 2.1 at 6
months and 10.5 mmol/L ±1.7 at 12 months from surgery, which reflects no statistically significant change over the follow-up period.

6.3.3.3 Branched-chain amino-acids
Total BCAA levels T2DM significantly reduced following bariatric surgery, both in participants with (p<0.001) and without T2DM (p=0.034) (Figure 6.7). Significant reductions from baseline were to 6 weeks and from baseline to 12 months were seen in both groups, as illustrated in Figure 6.7.

Figure 6.7: BCAA levels before and after bariatric surgery in people with and without T2DM

Figure illustrating BCAA levels before and after bariatric surgery in participants without (A) and with T2DM (B). A: In participants without T2DM baseline levels of BCAA were 0.41mmol/L ±0.08, which reduced to 0.29mmol/L ±0.05 at 6 weeks, 0.34 mmol/L ±0.07 at 6 months and 0.33 mmol/L ±0.04 at 12 months (p<0.001). This reflects significant reductions from baseline to 6 weeks (mean difference 0.12mmol/L, CI 0.06 to 0.18, p<0.001) and from baseline to 6 months (mean difference 0.07mmol/L, CI 0.02 to 0.13, p=0.007). B: In participants with T2DM baseline levels of BCAA were 0.27mmol/L ±0.17, which reduced to 0.22mmol/L ±0.15 at 6 weeks, 0.24 mmol/L ±0.07 at 6 months and 0.22mmol/L ±0.04 at 12 months (p=0.034). This reflects significant reductions from baseline to 6 weeks (mean difference 0.05mmol/L, CI 0.01 to 0.09, p=0.038) and from baseline to 6 months (mean difference 0.05mmol/L, CI 0.01 to 0.10, p=0.012). (* indicates p<0.05, ** p<0.01 and *** p<0.001). BCAA: Branched-chain Amino Acids, T2DM: Type 2 Diabetes Mellitus.

6.3.4 Biomarkers relevant to infectious disease risk
Results for a series of 25 biomarkers identified as relevant for the risk prediction of severe illness in the context of infectious disease were compared prior to and at 6
weeks, 6 months and 12 months from bariatric surgery in participants without T2DM (Table 6.4) and with T2DM (Table 6.5) respectively.

Significant changes following surgery were identified in people without T2DM, following adjustment for multiple comparisons, in circulating levels of GlycA (p<0.0001), creatinine (p=0.0002), glucose (p=0.0018), tyrosine (p<0.0001), phenylalanine (p<0.0001), glycine (p=0.0002), valine (p<0.0001), leucine (p=0.0001), as well as omega-6 % (p=0.0003) and the apolipoprotein B/apolipoprotein A1 ratio (p<0.0001) (Table 6.4).

In people with T2DM, bariatric surgery led to significant changes in circulating levels of GlycA (p<0.0001), glucose(p<0.0001), lactate(p=0.0006), valine (p=0.0002) and the omega-6/omega-3 ratio (p=0.0007) (Table 6.5).

<table>
<thead>
<tr>
<th>Table 6.4: Metabolomic biomarkers in participants without T2DM</th>
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<tbody>
<tr>
<td><strong>Baseline</strong></td>
</tr>
<tr>
<td>GlycA (mmol/L)</td>
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<td>DHA %</td>
</tr>
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<td>Creatinine</td>
</tr>
<tr>
<td>MUFA</td>
</tr>
<tr>
<td>Apolipoprotein B / Apolipoprotein A1</td>
</tr>
<tr>
<td>Tyrosine</td>
</tr>
<tr>
<td>Isoleucine</td>
</tr>
<tr>
<td>SFA %</td>
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<tr>
<td></td>
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</tr>
<tr>
<td><strong>Glucose</strong></td>
</tr>
<tr>
<td><strong>Lactate</strong></td>
</tr>
<tr>
<td><strong>Omega-6/Omega-3</strong></td>
</tr>
<tr>
<td><strong>Phenylalanine</strong></td>
</tr>
<tr>
<td><strong>Total cholesterol</strong></td>
</tr>
<tr>
<td><strong>Omega-6 %</strong></td>
</tr>
<tr>
<td><strong>Alanine</strong></td>
</tr>
<tr>
<td><strong>PUFA</strong></td>
</tr>
<tr>
<td><strong>Glycine</strong></td>
</tr>
<tr>
<td><strong>Histidine</strong></td>
</tr>
<tr>
<td><strong>PUFA %</strong></td>
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<tr>
<td><strong>Valine</strong></td>
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<tr>
<td><strong>Leucine</strong></td>
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<td><strong>Albumin</strong></td>
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<tr>
<td><strong>Omega-3</strong></td>
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<tr>
<td><strong>LDL cholesterol</strong></td>
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<tr>
<td><strong>Triglycerides</strong></td>
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Results for levels of 25 metabolic biomarkers identified as relevant for the risk of severe illness from infectious disease by Julkunen et al., prior to, at 6 week, 6 months and 12 months following bariatric surgery[19] in participants without T2DM. The significance level was adjusted for multiple comparisons using a Bonferroni to 0.002 DHA: docosahexaenoic acid; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids; SFA: saturated fatty acids. Sig: Significance
<table>
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<tr>
<th>Metabolomic Biomarkers</th>
<th>Baseline</th>
<th>6 weeks</th>
<th>6 months</th>
<th>12 months</th>
<th>P value</th>
<th>Sig</th>
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<td>DHA %</td>
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<td>Lactate</td>
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<td>Omega-6/Omega-3</td>
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<td>9.8 ±2.6</td>
<td>11.3 ±3.0</td>
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<td>Phenylalanine</td>
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<td>Omega-6 %</td>
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<td>Alanine</td>
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<td>Glycine</td>
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<td>Histidine</td>
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<td>0.1561 NS</td>
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</table>
Results for levels of 25 metabolic biomarkers identified as relevant for the risk of severe illness from infectious disease by Julkunen et al., prior to, at 6 weeks, 6 months and 12 months following bariatric surgery in people with T2DM[19]. The significance level was adjusted for multiple comparisons using a Bonferroni to 0.002. DHA: docosahexaenoic acid; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids; SFA: saturated fatty acids. Sig: Significance.

### 6.3.5 Infectious disease score

Using the weighting coefficients from the infectious disease score devised by Julkunen et al., a score was calculated for each participant based on their results from the 25 biomarkers (Table 6.1), for participants with and without T2DM respectively. The mean score was 8.2 ±2.8 in participants without T2DM and 5.1 ±2.6 in participants with T2DM (p=0.002).

In participants without T2DM, the score at baseline was calculated at 8.2 ±2.8. At 6 weeks following bariatric surgery, this reduced to 5.1 ±2.4, at 6 months to 4.7 ±2.1 and at 12 months to 4.7 ±1.8, representing a significant reduction from baseline to 6 weeks (p<0.001), baseline to 6 months (p<0.001) and baseline to 12 months (p<0.001).

In participants with T2DM the baseline score before bariatric surgery was 5.1 ±2.6. At 6 weeks following bariatric surgery the score was calculated at 5.3 ±2.2.4. At 6 months, the score dropped to 4.1 ±2.5 and at 12 months from surgery to 3.9 ±2.0. ANOVA repeated measures analysis showed significant differences from baseline (p=0.043),

<table>
<thead>
<tr>
<th></th>
<th>40.3 ±2.9</th>
<th>41.1 ±2.3</th>
<th>41.6 ±2.3</th>
<th>42.7 ±1.9</th>
<th>0.0032</th>
<th>NS</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>PUFA %</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Valine</strong></td>
<td>0.247 ±0.033</td>
<td>0.199 ±0.034</td>
<td>0.213 ±0.044</td>
<td>0.201 ±0.028</td>
<td>0.0002</td>
<td>**</td>
</tr>
<tr>
<td><strong>Leucine</strong></td>
<td>0.116 ±0.017</td>
<td>0.091 ±0.03</td>
<td>0.097 ±0.023</td>
<td>0.094 ±0.015</td>
<td>0.0121</td>
<td>NS</td>
</tr>
<tr>
<td><strong>Albumin</strong></td>
<td>40.2 ±2.8</td>
<td>38.7 ±3.2</td>
<td>37.7 ±2.5</td>
<td>37.5 ±3.3</td>
<td>0.0082</td>
<td>NS</td>
</tr>
<tr>
<td><strong>Omega-3</strong></td>
<td>0.470 ±0.138</td>
<td>0.506 ±0.213</td>
<td>0.455 ±0.152</td>
<td>0.383 ±0.095</td>
<td>0.0977</td>
<td>NS</td>
</tr>
<tr>
<td><strong>LDL cholesterol</strong></td>
<td>1.6 ±0.4</td>
<td>1.5 ±0.5</td>
<td>1.6 ±0.5</td>
<td>1.5 ±0.4</td>
<td>0.6325</td>
<td>NS</td>
</tr>
<tr>
<td><strong>Triglycerides</strong></td>
<td>1.2 ±0.7</td>
<td>1.0 ±0.4</td>
<td>1.0 ±0.4</td>
<td>0.9 ±0.4</td>
<td>0.1070</td>
<td>NS</td>
</tr>
</tbody>
</table>
however, following adjustment for multiple comparisons the drop from baseline to 12 months was no longer significant (p=0.103).

### 6.3.6 Correlation of adipocytokine levels to BMI and %WL

In order to investigate the presence of any potential correlations between adipocytokine levels and baseline BMI, as well as their change following bariatric surgery to post-operative %WL, Pearson’s correlation analyses were undertaken. Table 6.6 summarises results for the cohort without T2DM and Table 6.7 for the group of participants with T2DM. At baseline, there were also no significant correlations identified between levels of GDF15, TNFa, IL-6, IL-10 GlycA or BCAA in people with or without T2DM.

Potential correlations between the levels of GDF15, TNFa, IL-6, IL-10 GlycA or BCAA were examined at baseline. No correlations were identified in participants without T2DM. In participants with T2DM at baseline, significant correlations were seen between TNFa and IL-6 levels (r 0.655, R² 0.428, CI 0.189 – 0.880, p=0.011) and GDF15 and GlycA levels (r 0.611, R² 0.373, CI 0.090 to 0.869, p=0.027). The relationship between baseline BMI and adiponectin was also examined and no significant correlation was identified for participants without T2DM (r -0.118, R² 0.014, CI -0.514 – 0.320, p=0.603) or with T2DM (r -0.127, R² 0.016, CI -0.616 – 0.432, p=0.666).

At 12 months from surgery no correlation was seen in people without T2DM between post-operative %WL and the change in GDF15, TNFa, IL-6, IL-10 GlycA or BCAA from baseline to 12 months (Table 6.8). Likewise, the rise in adiponectin at 12 months post-surgery did not correlate with %WL in participants without T2DM (r -0.228, R² 0.015, CI -0.518 – 0.316, p=0.587).

In participants with T2DM, %WL correlated with the change in IL-10 levels (r -0.538, R² 0.290, CI-0.831 to -0.011, p=0.047) (Table 6.9) and with the change in adiponectin levels (r 0.615, R² 0.378, CI 0.125 – 0.864, p=0.020).
In addition, potential correlations between the change in levels of GDF15, TNFa, IL-6, IL-10, adiponectin GlycA and BCAA from baseline to 12 months were investigated. In people without T2DM, there was a significant correlation in the change from baseline to 12 months between levels of IL-6 and IL-10 (r 0.933, R² 0.871, IC -0.972 to -0.844, p<0.001). In people with T2DM instead, a significant correlation was seen between the change from baseline to 12 months between GDF15 and TNFa levels (r -0.654, R² -0.086 to -0.161, p=0.015).

Table 6.6: Pearson’s correlation analyses between levels of GDF15, TNFa, IL-6, IL-10, GlycA and BCAA and baseline BMI in participants without T2DM

<table>
<thead>
<tr>
<th></th>
<th>BMI vs. GDF15</th>
<th>BMI vs. TNFa</th>
<th>BMI vs. IL-6</th>
<th>BMI vs. IL-10</th>
<th>BMI vs. GlycA</th>
<th>BMI vs. BCAA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pearson r</td>
<td>0.284</td>
<td>0.135</td>
<td>-0.264</td>
<td>0.196</td>
<td>0.063</td>
<td>-0.153</td>
</tr>
<tr>
<td>95% CI</td>
<td>-0.156 to 0.630</td>
<td>-0.304 to 0.527</td>
<td>-0.617 to 0.178</td>
<td>-0.246 to 0.570</td>
<td>-0.368 to 0.472</td>
<td>-0.540 to 0.207</td>
</tr>
<tr>
<td>R²</td>
<td>0.081</td>
<td>0.018</td>
<td>0.069</td>
<td>0.038</td>
<td>0.004</td>
<td>0.023</td>
</tr>
<tr>
<td>p value</td>
<td>0.200</td>
<td>0.548</td>
<td>0.236</td>
<td>0.383</td>
<td>0.779</td>
<td>0.497</td>
</tr>
</tbody>
</table>


Table 6.7: Pearson’s correlation analyses between levels of GDF15, TNFa, IL-6, IL-10, GlycA and BCAA and baseline BMI in participants with T2DM

<table>
<thead>
<tr>
<th></th>
<th>BMI vs. GDF15</th>
<th>BMI vs. TNFa</th>
<th>BMI vs. IL-6</th>
<th>BMI vs. IL-10</th>
<th>BMI vs. GlycA</th>
<th>BMI vs. BCAA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pearson r</td>
<td>0.456</td>
<td>-0.136</td>
<td>0.094</td>
<td>0.001</td>
<td>0.296</td>
<td>0.343</td>
</tr>
<tr>
<td>95% CI</td>
<td>-0.122 to 0.806</td>
<td>-0.622 to 0.425</td>
<td>-0.459 to 0.595</td>
<td>-0.530 to 0.531</td>
<td>-0.278 to 0.715</td>
<td>-0.230 to 0.739</td>
</tr>
<tr>
<td>R²</td>
<td>0.211</td>
<td>0.019</td>
<td>0.009</td>
<td>&lt;0.001</td>
<td>0.0877</td>
<td>0.117</td>
</tr>
<tr>
<td>p value</td>
<td>0.114</td>
<td>0.642</td>
<td>0.748</td>
<td>0.999</td>
<td>0.304</td>
<td>0.231</td>
</tr>
</tbody>
</table>

Table 6.8: Pearson’s correlation analyses between post-operative 12 month %WL and the change from baseline (delta) in GDF15, TNFa, IL-6, IL-10, GlycA and BCAA levels in participants without T2DM

<table>
<thead>
<tr>
<th></th>
<th>%WL vs. GDF15 delta</th>
<th>%WL vs. TNFa delta</th>
<th>%WL vs. IL-6 delta</th>
<th>%WL vs. IL-10 delta</th>
<th>%WL vs. GlycA delta</th>
<th>%WL vs. BCAA delta</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pearson r</td>
<td>-0.110</td>
<td>0.001</td>
<td>0.142</td>
<td>0.238</td>
<td>0.177</td>
<td>0.075</td>
</tr>
<tr>
<td>95% CI</td>
<td>-0.508 to -0.421</td>
<td>-0.298 to 0.531</td>
<td>-0.204 to 0.599</td>
<td>-0.265 to 0.557</td>
<td>-0.358 to 0.482</td>
<td></td>
</tr>
<tr>
<td>R²</td>
<td>0.012</td>
<td>&lt;0.001</td>
<td>0.020</td>
<td>0.057</td>
<td>0.031</td>
<td>0.006</td>
</tr>
<tr>
<td>p value</td>
<td>0.627</td>
<td>0.999</td>
<td>0.530</td>
<td>0.287</td>
<td>0.432</td>
<td>0.739</td>
</tr>
</tbody>
</table>


Table 6.9: Pearson’s correlation analyses between post-operative 12 month %WL and the change from baseline (delta) in GDF15, TNFa, IL-6, IL-10, GlycA and BCAA levels in participants with T2DM

<table>
<thead>
<tr>
<th></th>
<th>%WL vs. GDF15 delta</th>
<th>%WL vs. TNFa delta</th>
<th>%WL vs. IL-6 delta</th>
<th>%WL vs. IL-10 delta</th>
<th>%WL vs. GlycA delta</th>
<th>%WL vs. BCAA delta</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pearson r</td>
<td>-0.261</td>
<td>0.234</td>
<td>-0.292</td>
<td>-0.538</td>
<td>-0.306</td>
<td>-0.426</td>
</tr>
<tr>
<td>95% CI</td>
<td>-0.710 to -0.339</td>
<td>-0.712 to 0.282</td>
<td>-0.831 to 0.011</td>
<td>-0.720 to 0.268</td>
<td>-0.780 to 0.135</td>
<td></td>
</tr>
<tr>
<td>R²</td>
<td>0.068</td>
<td>0.055</td>
<td>0.085</td>
<td>0.290</td>
<td>0.094</td>
<td>0.181</td>
</tr>
<tr>
<td>p value</td>
<td>0.389</td>
<td>0.421</td>
<td>0.311</td>
<td>0.047</td>
<td>0.287</td>
<td>0.129</td>
</tr>
</tbody>
</table>

6.3.7 Interquartile ranges for pro-inflammatory adipocytokines

In order to identify participants who may be expressing a more pro-inflammatory phenotype at baseline, the interquartile range was calculated for baseline circulating levels of GDF15, TNFa, IL-6, BCAA and GlycA measured in participants with and without T2DM respectively. Interquartile ranges for participants without T2DM and with T2DM can be seen in Table 6.10 and Table 6.12 respectively. Participants’ results were then ranked based on the quartile they were in and for each of their results individually and
for all their results combined, as shown for participants without T2DM in Table 6.11 and for participants with T2DM in Table 6.13. Tables 6.11 and 6.13 respectively, highlight participants with a higher versus lower inflammatory profile, based on the quartiles their results ranked in for each of the selected adipocytokines and biomarkers.

Table 6.10: Interquartile range for GDF15, TNFa, IL-6, GlycA and BCAA levels measured at baseline in participants without T2DM

<table>
<thead>
<tr>
<th></th>
<th>GDF15 (pg/mL)</th>
<th>TNFa (pg/mL)</th>
<th>IL-6 (pg/mL)</th>
<th>GlycA (mmol/mL)</th>
<th>BCAA (mmol/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1st quartile</td>
<td>356.4</td>
<td>7.4</td>
<td>1.9</td>
<td>0.895</td>
<td>0.360</td>
</tr>
<tr>
<td>2nd quartile</td>
<td>560.1</td>
<td>10.7</td>
<td>3.5</td>
<td>0.990</td>
<td>0.390</td>
</tr>
<tr>
<td>3rd quartile</td>
<td>732.5</td>
<td>15.5</td>
<td>5.1</td>
<td>1.110</td>
<td>0.418</td>
</tr>
<tr>
<td>Interquartile range</td>
<td>356-560.1</td>
<td>7.4-15.5</td>
<td>1.9-3.5</td>
<td>0.895-1.110</td>
<td>0.360-0.418</td>
</tr>
</tbody>
</table>

GDF15: Growth differentiation factor 15, TNF: Tumour Necrosis Factor alpha, IL-6: Interleukin 6, IL-10: Interleukin 10, GlycA: Glyceroprotein Acetyl, BCAA: Branched-Chain Amino Acids, CI: Confidence Interval.
Table 6.11: Quartile for GDF15, TNFa, IL-6, GlycA and BCAA levels measured at baseline per participant without T2DM

<table>
<thead>
<tr>
<th></th>
<th>GDF15</th>
<th>TNFa</th>
<th>IL-6</th>
<th>GlycA</th>
<th>BCAA</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Participant 1</td>
<td>2</td>
<td>4</td>
<td>4</td>
<td>4</td>
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<tr>
<td>Participant 2</td>
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<td>Participant 3</td>
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<tr>
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</table>

GDF15: Growth differentiation factor 15, TNF: Tumour Necrosis Factor alpha, IL-6: Interleukin 6, IL-10: Interleukin 10, GlycA: Glyceroprotein Acetyl, BCAA: Branched-Chain Amino Acids, CI: Confidence Interval.
Table 6.12: Interquartile range for GDF15, TNFa, IL-6, GlycA and BCAA levels measured at baseline in participants with T2DM

<table>
<thead>
<tr>
<th></th>
<th>GDF15f (pg/mL)</th>
<th>TNFa (pg/mL)</th>
<th>IL-6 (pg/mL)</th>
<th>GlycA (mmol/mL)</th>
<th>BCAA (mmol/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1st quartile</td>
<td>657.6</td>
<td>6.0</td>
<td>2.3</td>
<td>0.859</td>
<td>0.111</td>
</tr>
<tr>
<td>2nd quartile</td>
<td>1121.6</td>
<td>7.9</td>
<td>2.8</td>
<td>0.927</td>
<td>0.242</td>
</tr>
<tr>
<td>3rd quartile</td>
<td>1547.8</td>
<td>12.2</td>
<td>3.9</td>
<td>1.048</td>
<td>0.430</td>
</tr>
<tr>
<td>Interquartile range</td>
<td>657.6-1547.8</td>
<td>6.0-12.2</td>
<td>2.3-3.9</td>
<td>0.859-1.048</td>
<td>0.111-0.430</td>
</tr>
</tbody>
</table>

Table 6.13: Quartile for GDF15, TNFa, IL-6, GlycA and BCAA levels measured at baseline per participant with T2DM

<table>
<thead>
<tr>
<th></th>
<th>GDF15</th>
<th>TNFa</th>
<th>IL-6</th>
<th>GlycA</th>
<th>BCAA</th>
<th>Total</th>
</tr>
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GDF15: Growth differentiation factor 15, TNF: Tumour Necrosis Factor alpha, IL-6: Interleukin 6, IL-10: Interleukin 10, GlycA: Glyceroprotein Acetyl, BCAA: Branched-Chain Amino Acids, CI: Confidence Interval.
6.4 Discussion

This study investigated levels of circulating adipocytokines and metabolomic biomarkers in people with obesity and illustrated how these are altered by bariatric surgery longitudinally at 6 weeks, 6 months and 12 months in a cohort with and one without T2DM. Participants included in each of the two cohorts in this study were comparable in terms of their age, sex, baseline BMI and were matched to procedure type and post-operative %WL in order to eliminate effects due to variability of response to surgery.

At baseline, levels of adiponectin, TNFa, IL-6 and IL-10 were comparable in people with and without T2DM. Likewise, there were no differences at baseline in GlycA levels and circulating fatty acid levels between the two study groups. Levels of GDF15 and BCAAs however were higher in people with T2DM. GDF15 is consistently reported to be higher in participants with T2DM, as reported in Chapter 4[28]. BCAA levels have been consistently reported to be higher in people with T2DM and have additionally been identified as a marker of insulin resistance[29-31]. Furthermore, although the causality of this relationship remains incompletely understood, BCAA are directly contribute to the pathogenesis of T2DM. The accumulation of toxic BCAA metabolites impairs beta-cell mitochondrial function, which eventually results in beta-cell apoptosis[32].

Interestingly, although the difference was not statistically significant, despite the associations between TNFa, insulin resistance and T2DM, TNFa levels in this study were higher in people without T2DM (12.8pg/mL ±7.2 vs. 8.8pg/mL ±4.0, p=0.097). This may in part be due to the high proportion of participants in the T2DM group being treated with metformin prior to bariatric surgery. Metformin has demonstrated anti-inflammatory quantities and treatment with metformin has been shown to reduce levels of both TNFa and IL-6[33,34].
6.4.1 Adipocytokine levels following bariatric surgery in people with and without T2DM

The data from this study demonstrated significant rises in adiponectin levels from baseline to 12 months post-bariatric surgery both in participant with and without T2DM. Significant elevations in plasma adiponectin have been shown previously both following RYGB and SG[35,36]. A meta-analysis additionally concluded that adiponectin levels increase significantly following bariatric surgery[36]. Most studies to date have either investigated the short- or the longer term effects of bariatric surgery on adiponectin levels[21,37,38]. Linscheid et al., previously showed ongoing elevations from 6-week to 12 months following surgery in a study of 5 patients following RYGB[39]. The novelty of the presented findings of this study therefore lies in illustrating the trend of change in adiponectin secretion over the study’s 12-month follow up period in our study populations. As illustrated in Figure 6.1, although rises in adiponectin occur early following surgery, the largest and most significant rises in adiponectin were seen at 12 months, suggesting that this is a later metabolic effect of bariatric surgery, compared to some of the immediate metabolic benefits seen, for instance, in gut hormone secretion profiles[40]. Interestingly, a study quantifying VAT mass at 6 months following RYGB demonstrated that rises in adiponectin correlated with reductions in VAT following surgery, suggesting a degree of adipose tissue mass reduction and remodelling may be required before the full effect of bariatric surgery on adiponectin secretion is evident[41].

No significant changes were demonstrated in circulating TNFa levels following bariatric surgery, neither in people with nor without T2DM. A series of studies have published results on TNFa levels following bariatric surgery with highly variable results. Although both increased[42,43] and decreased[22,44,45] circulating levels of TNFa have been reported by some studies, the majority of the studies report no significant changes[25,46-48]. Although the results in our study were not statistically significant, Figure 6.2 does illustrate a differential secretion profile following surgery in people with and without T2DM. In participants without T2DM, a transient rise was seen at 6 weeks from surgery with levels returning to baseline at 6 and 12 months. In participants without T2DM, TNFa levels rose following bariatric surgery. Considering the early post-
operative period following bariatric surgery is highly catabolic and a period of active lipolysis, elevations in TNFa at 6 weeks post-surgery are not surprising[49]. A study investigating TNFa expression in SAT samples collected during and 1 year following bariatric surgery showed increased TNFa expression combined with downregulation of other pro-inflammatory cytokines such as IL-6 and IL-1b, concluding that the TNFa upregulation was an indicator for increased lipolysis and adipose tissue catabolism[50]. Interestingly, in a study comparing systemic versus adipocyte insulin sensitivity in people with T2DM following RYGB demonstrated that although systemic insulin sensitivity drastically improved in the immediate post-operative period, there were no changes in adipocyte insulin sensitivity at 4 weeks following surgery, despite a marked reduction in adipocyte size[51]. With regard to the TNFa elevations in participants with T2DM, it is worth considering if pre-existing chronic inflammation is more established in this cohort and in addition, whether withdrawal of metformin in the immediately post-operative period may additionally impact upon adipose tissue catabolism.

In this study, the post-operative response in circulating IL-6 levels was markedly different to TNFa. A gradual reduction was seen following surgery over the study’s follow up period in participants without T2DM, with a significant reduction at 12 months from surgery. In participants with T2DM, a significant reduction was seen from baseline to 6 months from surgery, with levels rising again at 12 months. Notably, the response within the T2DM group was much more homogenous, as evidenced by the considerably lower SD. The effects of bariatric surgery on IL-6 have been investigated in a number of studies. The majority of these report either a decrease[44,45,52] or unchanged circulating IL-6 levels following surgery[42,48,53]. These findings, combined with the fact that IL-6 is downregulated in adipose tissue following bariatric surgery support the notion that the reduction in IL-6 following bariatric surgery is a manifestation of an improvement in a chronic obesity-associated inflammation.

The literature surrounding IL-10, its roles and the effect of bariatric surgery upon its secretion remains largely controversial. In this study, IL-10 levels following bariatric surgery did not significantly change. In the group of participants with T2DM, a reduction at 12 months following surgery was seen, which was no longer significant
when adjusting for multiple comparisons. Despite the fact however that the changes following bariatric surgery in this study were not statistically significant, the trend illustrates an overall reduction in the IL-10 levels at 12 months from surgery. Studies reporting IL-10 levels after bariatric surgery to date are limited and have either shown no difference[54] or an increase in circulating IL-10 levels[55,56]. Although our findings are in contrast to these observations, taking into consideration the facts that increased IL-10 levels have been measured in people with obesity compared to normal weight individuals and the association between IL-10 elevation an COVID-19 severity, this reduction, may in fact represent an improvement in an inflammatory state in a subset of individuals[9,57].

GDF15 levels were also measured in the two subgroups of participants before and after surgery. GDF15 levels reduced following a transient elevation in participants without T2DM. In participants with T2DM levels gradually reduced, with a significant drop from baseline at 12 months from surgery. These observations are in keeping with the results presented in Chapter 4, although there is an overlap of some participants who were included in both studies. Irrespective of any potential effects of circulating GDF15 on appetite in people with obesity and following bariatric surgery, a substantial body of evidence suggests that the persistent elevation in GDF15 in people with obesity, and even more so in people with obesity and T2DM, is a manifestation of a systemic low-grade inflammatory state[58,59]. In addition, the observation from animal studies that GDF15 is produced in response to activating the cellular integrated stress response in the context of over-nutrition provides substantial evidence to support the link between GDF15 over-secretion, inflammation and obesity[59]. Therefore, assuming GDF15 is a manifestation of inflammation, reductions following bariatric surgery in people with pre-surgery over-secretion is an expected finding implying an improvement in obesity-associated physiology.

Interpreting the change in adipocytokine levels in response to weight loss interventions in the context of the controversies in the literature poses several challenges. Considerable variabilities evidently underlie the populations of participants included in these studies. Additionally, given the absence of existing established criteria to identify
people with ongoing obesity-associated inflammation, the findings of these studies will largely depend on the inflammatory characteristics of the participants recruited into them. One obvious finding in this study, is the variability of the levels of TNFa, IL-6, IL-10 and recorded at the baseline visit in our study populations, particularly in the group without T2DM. A subset of participants in this group displayed significant elevations in TNFa and IL-6 at baseline. Furthermore, both TNFa and IL-6 were higher in participants without T2DM, a group of patients which in clinical settings may be characterised as lower risk, particularly in absence of co-morbidities. With these observations in mind, it is important to note the findings we observed when dividing the study cohort into participants with a post-bariatric surgery reduction in these cytokines versus a post-operative rise. Participants without T2DM with a reduction in TNFa, IL-10 and GDF15 at 12 months from surgery had significantly higher levels of these cytokines at baseline. The same trend was observed for IL-6 but this did not reach statistical significance. These findings suggest that bariatric surgery reduces levels of underlying inflammation, when this is present. The effect of bariatric surgery however on adipocytokines in people with lower baseline inflammation remains unknown. Interestingly, the same effect was not seen in participants with T2DM, suggesting that the changes in these adipocytokines following bariatric surgery are more likely to be more closely related to improvements in glycaemia and insulin sensitivity[51].

6.4.2 Metabolomic biomarkers following bariatric surgery in people with obesity with and without T2DM

Metabolomic profiling was also used in this study in order to investigate the effects of bariatric surgery on biomarkers that have been linked to inflammation, insulin resistance and worse outcomes in COVID-19[19]. Following bariatric surgery, GlycA levels gradually reduced following bariatric surgery both in participants with and without T2DM, although the effect was more pronounced in the group without T2DM. GlycA is a marker of systemic inflammation with lower inter-individual variability compared to other biomarkers[60]. Two recent studies to date, have investigated GlycA levels before RYGB and SG and showed significant reductions following surgery[61,62]. These findings together suggest that bariatric surgery leads to improvement in systemic inflammation and oxidative stress[62]. Similarly, BCAA levels
reduced following surgery both in people with and without T2DM. A number of studies have shown reductions in BCAA levels following bariatric surgery[63-65]. Interestingly, large scale studies correlating metabolomic profiling to VAT mass, have shown a strong association between VAT and circulating BCAA even when correcting for glycaemia and circulating lipid levels[30]. For instance, glutamate, a by-product of BCAA catabolism, strongly correlates with VAT mass, whereas there are no associations between glutamate and SAT[66]. In line with those findings, the reduction in BCAA following bariatric surgery may reflect a reduction in VAT. The suggestion from current research that BCAA levels may be a surrogate marker for VAT may therefore be highly significant, as they open up the possibility of BCAA measurement being used as a non-invasive marker to quantify a person's VAT mass and associated risks, without the need for labour-intensive detailed imaging studies[17,18].

Parallels in circulating levels of adiponectin, GlycA, BCAA, IL-6, IL-10 and GDF15 following bariatric surgery were seen, with regard to the fact that the most marked change from baseline was demonstrated at 12 months from surgery. This may suggest that resolution of the pro-inflammatory effects of bariatric surgery may be a slightly later effect, compared to some of the immediate benefits seen with regards to appetite and T2DM resolution[40,67]. Given the catabolic state of the immediate post-operative period, with active lipolysis and adipose tissue remodelling, releasing fatty acids into the systemic circulation, it would not be unexpected for these processes to result in a degree of inflammatory responses[68].

In this study, the effect of bariatric surgery on a subset of metabolomic biomarkers comprising an infectious disease score, linked to an increased risk of severe pneumonia and severe COVID-19 was investigated[19]. Improvements on a number of these markers were observed, reflecting the improvements in inflammation, BCAA described above, as well markers of lipid metabolism. Although no significant changes were seen in total fatty acid levels following surgery, this was likely masked by changes in the different subclasses of fatty acids. Subsequently, we calculated a score based on the formula published by Julkunen et al., however using absolute values of measured biomarkers in order to enable comparison between the different time follow-up time
Significant reductions in the calculated score values were evident in participants without T2DM. Importantly, these were already present at 6-weeks from surgery, with further reduction seen at 6 months and maintained at 12 months (p<0.001). Although these results are exploratory and will require validation, taken together with the cytokine findings discussed earlier, may suggest that although the adipose tissue remodelling seen during the periods of rapid weight loss following bariatric surgery is likely to trigger some inflammatory changes _per se_, the overall impact of bariatric surgery on systemic inflammation is already beneficial during at 6 weeks from surgery. Interestingly however, the same degree of reduction in the infectious disease score was not observed in participants with T2DM. This may be in part due to the fact that their baseline score was lower, reflecting a lower risk population despite the presence of T2DM, as also evidenced by the lower baseline TNFa and IL-6 levels. Additional factors, directly related to insulin sensitivity and T2DM, as well as T2DM treatments, may also impact on this finding.

6.4.3 Correlation analyses between studied biomarkers and weight outcomes in people with and without T2DM

The results from the correlation analyses showed no significant relationship between BMI at baseline and any of the recorded adipocytokines. This observation is likely due to the narrow BMI ranges in the participants included in this study. In participants with T2DM at baseline TNFa and IL-6 levels positively correlated, which has been described in the literature before [69]. To our knowledge, GDF15 and GlycA have not been studied in conjunction before, but the correlation may represent a reflection of systemic inflammation. Correlation analyses between the change in measured adipocytokines and %WL showed no significant correlations in people without T2DM. In people with T2DM, %WL correlated with the change in IL-10 and the change in adiponectin. Adiponectin changes have previously been correlated with the reduction in VAT which is likely to explain this finding [41]. Overall however, the lack of correlations between the studied adipocytokines, baseline BMI and post-operative %WL, suggest that BMI is a poor marker of both ongoing inflammation in the context of obesity and %WL a poor indicator of the impact a weight loss intervention has upon chronic systemic inflammation. These findings therefore highlight the fact that more accurate and
prognostic markers beyond weight loss and additional classification methods for obesity beyond BMI are needed.

6.4.4 Interquartile ranges and pro-inflammatory phenotyping

The interquartile ranges for each of the pro-inflammatory cytokines in measured in this study were calculated and participants with and without T2DM ranked from the highest to the lowest levels. To date, there are no available diagnostic criteria to identify people with obesity-associated chronic inflammation. In addition, there are no accepted risk-stratification tools to enable identification of people with obesity at an increased risk of cardiometabolic disease nor of developing severe illness in the context of infection. These exploratory data shown in this part of the study, highlight the wide range of measured pro-inflammatory cytokines in an otherwise very homogenous cohort of participants with severe obesity. In current clinical settings, there are no tools to identify or prioritise treatment, for instance in participants 1-5 highlighted in Table 6.11, who display elevated levels of a series of pro-inflammatory cytokines, all linked to a higher risk of severe disease and poor outcomes in COVID-19. Further studies enabling risk-related phenotyping in a manner translatable to clinical settings, would have significant implications in other to protect and prioritise treatment in people with obesity and associated chronic inflammation.

6.4.5 Limitations

The data presented in this study are exploratory and have a number of limitations. The study evaluates the impact of a single method of treatment, bariatric surgery, with no control group or comparison to other means of treatment, such as pharmacotherapy or lifestyle interventions. Importantly, no body composition data to allow for quantification of VAT mass and correlation to levels of adipocytokines and metabolomic biomarkers. Furthermore, participants in this study were exclusively female, as females represent the majority of the population undergoing bariatric surgery at UCLH. Although due to sex-related differences in underlying physiology, the study’s findings may not be applicable to male cohorts, the single-sex recruitments allowed for a homologous cohort of participants to be studies, restricting additional confounding factors.
6.4.6 Future work
A series of future studies will be required in order to devise clinically applicable diagnostic and prognostic tools for obesity-related inflammation and associated clinical risks both for the development of cardiometabolic risk, as well as severe illness in the context of infection. Body composition studies using imaging modalities to locate and quantify ectopic and VAT, combined with tissue sampling, will be needed in order to determine the origin of the relevant inflammatory mediators and enable development and validation of accurate surrogate markers of VAT mass. Large cohorts with population-wide representation will also be required in order to enable development of diagnostic and prognostication tools that can be rolled out to clinical settings. Finally, more data on efficacy of different treatment modalities will be required, such as the novel GLP-1 analogue semaglutide, as well as mechanistic studies to investigate the potential role of immunotherapies[70,71].

6.5 Conclusions
The exploratory results presented in this chapter, highlight the wide range of inflammatory adipocytokines measured in a homogenous cohort of people with severe obesity. The findings of this study demonstrate improvements in multiple markers of inflammation, such as adiponectin, IL-6, GlycA and BCAA levels 12 months following bariatric surgery. Importantly, this study highlights that the most marked benefit with regards to improvement in markers related to inflammation, occurs in people with higher circulating levels of risk-associated adipocytokines at baseline. Furthermore, our findings highlight that BMI and %WL are poor indicators of both the degree of underlying chronic inflammation and its improvement following bariatric surgery. The findings from the work in this chapter, as well as chapter 4, suggest that it is not possible to draw generalised conclusions on the effect of bariatric surgery or obesity treatments on secretion profiles of pro-inflammatory adipocytokines without taking into consideration the characteristics of the patient population studied. The current literature as well as the findings of the work in this chapter suggest that there is considerable variability in cytokine profiles both at baseline and in response to bariatric surgery and that response to bariatric surgery depends on the degree of pre-existing
inflammation. Therefore, adequate studies that will lead to the development of diagnostic and prognostic tools for obesity-associated inflammation are urgently required in prioritise treatment for the people at the highest risk, which the COVID-19 pandemic has highlighted is necessary to protect people with obesity from adverse outcomes in the context of infection.
6.6 Chapter 6 References


APPENDIX: PUBLICATIONS
Seroprevalence of SARS-CoV-2 antibodies in people with an acute loss in their sense of smell and/or taste in a community-based population in London, UK: An observational cohort study

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Abstract

Background

Loss of smell and taste are commonly reported symptoms associated with coronavirus disease 2019 (COVID-19); however, the seroprevalence of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) antibodies in people with a acute loss of smell and/or taste is unknown. The study aimed to determine the seroprevalence of SARS-CoV-2 antibodies in a community-based population with an acute loss of smell and/or taste and to compare the frequency of COVID-19 associated symptoms in participants with and without SARS-CoV-2 antibodies. It also evaluated whether smell or taste loss are indicative of COVID-19 infection.

Methods and findings

Text messages, sent via primary care centers in London, United Kingdom, invited people with loss of smell and/or taste in the preceding month, to participate. Recruitment took place between 23 April 2020 and 14 May 2020. A total of 590 participants enrolled via a web-based platform and responded to questions about loss of smell and taste and other COVID-19 related symptoms. Mean age was 39.4 years (SD ± 12.0) and 69.1% (n = 392) of participants were female. A total of 567 (96.1%) had a telemedicine consultation during which their COVID-19 related symptoms were verified and a lateral flow immunoassay test that detected SARS-CoV-2 immunoglobulin G (IgG) and immunoglobulin M (IgM) antibodies was undertaken under medical supervision. A total of 77.6% of 567 participants with acute smell and/or taste loss had SARS-CoV-2 antibodies; of these, 39.8% (n = 175) had neither cough nor fever. New loss of smell was more prevalent in participants with SARS-CoV-2
Antibodies, compared with those without antibodies (93.4% versus 78.7%, \( p < 0.001 \)), whereas taste loss was equally prevalent (90.2% versus 89.0%, \( p = 0.738 \)). Seropositivity for SARS-CoV-2 was 3 times more likely in participants with smell loss (OR 2.86; 95% CI 1.27–6.36; \( p < 0.001 \)) compared with those with taste loss. The limitations of this study are the lack of a general population control group, the self-reported nature of the smell and taste changes, and the fact our methodology does not take into account the possibility that a population subset may not seroconvert to develop SARS-CoV-2 antibodies post-COVID-19.

Conclusions
Our findings suggest that recent loss of smell is a highly specific COVID-19 symptom and should be considered more generally in guiding case isolation, testing, and treatment of COVID-19.

Trials registration
ClinicalTrials.gov NCT04377815

Author summary

Why was this study done?

- Coronavirus disease 2019 (COVID-19), an infectious disease caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) virus, was declared a pandemic in March 2020.
- COVID-19 can cause loss or reduced ability to smell (anosmia) or taste, without cough or fever, but few countries recommend self-isolation and testing on the basis of smell or taste changes alone.
- This study aimed to find out the proportion of people who have developed SARS-CoV-2 antibodies in a community-based population with a newly developed loss in their sense of smell and/or taste in London, UK.

What did the researchers do and find?

- Text messages were sent out to people registered with a number of primary care centers in London inviting people with a new loss in their sense of smell and/or taste to participate.
- Recruited participants completed online questionnaires regarding demographics, their loss of smell and/or taste, and other COVID-19 symptoms, before they had a telemedicine consultation with a healthcare professional who confirmed the history of their symptoms and supervised a test to find out if they had SARS-CoV-2 antibodies.
- A total of 78% of 567 people with smell and/or taste loss had SARS-CoV-2 antibodies; of these, 40% had neither cough nor fever, and participants with loss of smell were 3 times more to have SARS-CoV-2 antibodies, compared with those with loss of taste.
What do these findings mean?

- Loss of smell is a highly specific symptom of COVID-19.
- COVID-19 can present with loss of smell and/or taste without cough or fever.
- Loss of smell should be taken into consideration in case isolation, testing, and treatment strategies for COVID-19.

Introduction

Coronavirus disease 2019 (COVID-19) is an acute infectious disease caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). COVID-19 has spread exponentially, with 27,417,497 cases and 894,241 deaths reported from 216 countries by 9 September 2020 [1]. In the absence of a vaccine and disease-specific treatments, strategies to contain the pandemic are focused on rapid case isolation and testing. Although originally described as a primarily respiratory disease, reports of COVID-19 presenting with multiple symptoms, including loss of smell and taste, emerged rapidly. Understanding the symptomatology of COVID-19 and the predictive value of symptoms is crucial for containment strategies. As lockdown policies ease globally, early recognition of COVID-19 symptoms by the public together with rapid self-isolation and testing will be of vital importance to limit disease spread.

Reports linking loss of the sense of smell and/or taste to COVID-19 emerged in March 2020 [2-4]. SARS-CoV-2 enters the human body via the angiotensin-converting enzyme-2 (ACE-2) receptor, highly expressed in the nasal epithelium [5]. Consequent inflammatory changes in the olfactory neuroepithelium could disrupt olfactory neuron function, leading to smell loss [6,7]. Thus, from a pathophysiological perspective, it is logical for COVID-19 to impact smell [5,6]. Smell and taste are highly interlinked, with an element of taste (flavor) perception mediated through retronasal olfaction; hence, loss of smell results in taste changes [6]. However, within the oral cavity, ACE-2 is highly expressed on tongue epithelial mucosal cells [3], raising the possibility that taste loss results from a direct effect of SARS-CoV-2 on the tongue and that taste loss alone could occur in the absence of smell loss.

Available data suggest a prevalence of smell and/or taste loss in the range of 31%–85% in COVID-19 patients [8-10]. In a prospective epidemiological study, 85.6% and 88.0% of patients with a polymerase chain reaction (PCR)-confirmed COVID-19 diagnosis reported a loss of their sense of smell and taste, respectively [9].

The largest data set of potential COVID-19 related symptoms stems from a web-based app that collected self-reported symptoms from 2,618,862 users in the United Kingdom (UK) and the United States (US). This included the question "Do you have a loss of smell/taste?”. The authors reported a strong association between loss of smell/taste and a diagnosis of COVID-19, and as a consequence, loss of smell/taste are now recognized presentations of COVID-19 in the UK [11,12]. Their methodology did not, however, enable them to differentiate between smell and taste loss [11,12].

The importance of acute loss of smell or taste, in isolation or combination, as a predictor of COVID-19 in a population presenting with chemo sensory symptoms is unknown. Currently, recommendations for self-isolation and testing based upon acute loss of smell/taste have only been adopted by a limited number of countries; the majority are focused on fever and respiratory symptoms (Table 1). We therefore set out to quantify the seroprevalence of SARS-CoV-2...
Table 1. Recognition of smell and/or taste loss as symptoms of COVID-19 in the 30 countries with the highest number of reported cases globally.

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<td>135,757</td>
<td>9,203</td>
<td>Yes</td>
<td>[26]</td>
</tr>
<tr>
<td>Bolivia</td>
<td>122,308</td>
<td>7,997</td>
<td>No</td>
<td>[27]</td>
</tr>
<tr>
<td>Qatar</td>
<td>120,579</td>
<td>205</td>
<td>No</td>
<td>[28]</td>
</tr>
<tr>
<td>Ecuador</td>
<td>110,757</td>
<td>16,627</td>
<td>No</td>
<td>[29]</td>
</tr>
<tr>
<td>Kazakhstan</td>
<td>106,498</td>
<td>1,634</td>
<td>No</td>
<td>[30]</td>
</tr>
</tbody>
</table>

1 Reported cases as per John Hopkins University of Medicine Coronavirus Resource Center [14], accessed 9 September 2020.

specific antibodies in a community-based cohort with a new loss of their sense of smell and/or taste during the COVID-19 outbreak in London, UK. Additionally, we compared the effect of isolated loss of smell and isolated loss of taste separately and then in combination and investigated whether new smell and/or tasteloss is indicative of COVID-19 in our study population.
Methods
Study design
The study was conducted in London, UK, and recruited between 23 April 2020 and 14 May 2020 at a time when loss of smell and/or taste were not recognized as COVID-19 symptoms. Recruitment was timed to capture people who experienced symptoms during the peak of the COVID-19 outbreak in London. Antibody testing was delivered using a telemedicine consultation. This approach was chosen to capture positive cases without the limitations of the time window restrictions of PCR and to enable testing and participation without face-to-face contact, reducing the infection risk to both participants and researchers.

Adults registered with 4 participating primary care centers in London were sent text messages to their mobile telephones inviting those who experienced a new loss of their sense of smell and/or taste in the preceding month to participate. The text message read: "Has your sense of smell or taste reduced in the last month? If yes and you'd like to be part of a COVID-19 research study go to [link]". Participants were then directed to an online platform (hosted by Dendrite Clinical Systems) with the study information and an eligibility check. Inclusion criteria were age ≥18 years, proficiency in written and spoken English, and access to video-calling. Exclusion criteria were any preexisting loss of the sense of smell or taste of longer than a month’s duration. Participation was voluntary, and written informed consent was obtained electronically. Enrolled participants completed an online questionnaire (see SI Text), capturing their sex, age, ethnicity, smoking status, previous COVID-19 testing, questions about their smell and taste symptoms, as well as other symptoms of COVID-19.

Participants were subsequently sent a point-of-care testing kit detecting the presence of immunoglobulin M (IgM) and immunoglobulin G (IgG) antibodies to SARS-CoV-2. A healthcare professional arranged a telemedicine video consultation with each participant. At the beginning of the consultation, they asked the participant to describe the changes that they had experienced in their sense of smell and/or taste. Participants were then supervised in performing the antibody test using a finger-prick sample of whole blood, and their results were discussed with them. Photographs of the test cassette were obtained and reviewed independently by a second healthcare professional to confirm the result. Participants with a prior COVID-19 PCR test result were offered antibody testing irrespective of their result. Testing was carried out between 24 April 2020 and 22 May 2020.

The antibody test used detected the presence of IgM and IgG antibodies to SARS-CoV-2 via lateral flow immunoassay (Wuhan UNiscience Biotechnology Co., Ltd. COVID-19 Antibody IgM/IgG) [15]. As part of the test’s validation, 1,585 cases were tested: 421 (positive) clinically confirmed (including PCR) COVID-19 patients and 1,164 controls. These showed that the product has a relative sensitivity of 98.8% (95% CI 97.3%–99.6%) and a relative specificity of 98.9% (95% CI 97.15%–98.7%) [15].

The study received ethical approval from the National Health Service Queen’s Square Research Ethics Committee (IRAS Project ID 282668, ClinicalTrials.gov NCT04377815) and was conducted in line with the declaration of Helsinki and Good Clinical Practice.

Statistical analysis
A sample size calculation was undertaken in order to determine the study’s recruitment target, using the information on reported symptoms from the web-based COVID symptom study app developed by King’s College London and symptom reporting between the 24–29 March 2020 [16]. To calculate an estimate of 50% (95% CI 45%–55%) as the proportion who would test positive amongst those who reported a change in smell or taste, we needed 385 participants in the study [17]. Assuming a 15% attrition, we needed 453 participants in the study.
recruitment target was set to 500 participants to allow for larger attrition and increase accuracy. This was exceeded to improve the accuracy of the data and enable additional analyses to further describe the patterns of loss of smell and/or taste in the study populations with and without SARS-CoV-2 antibodies. Data were analyzed using GraphPad Prism version 8 (https://www.graphpad.com/scientific-software/prism/) and STATA version 15 (https://www.stata-uk.co.uk/). Data analysis was planned on completion of SARS-CoV-2 antibody testing. There was no prospective study protocol or analysis plan, and no data-driven changes to analyses took place. Descriptive analyses included the calculation of means (plus standard deviation [SD]) for continuous variables and numbers (n, with percentages) for categorical variables. Chi-squared tests were performed on categorical data as part of the secondary analysis. The significance level was adjusted for multiple comparisons by applying a Bonferroni correction when comparing symptoms other than loss of smell or taste. Logistic regression analysis was performed to estimate the association between loss of smell and/or taste and the presence of SARS-CoV-2 antibodies.

Results

Study population
A total of 33,650 text messages were sent out to people registered with 4 participating primary care centers. A total of 650 participants completed the registration process; 60 participants were ineligible and excluded. The participant flowchart [18] is illustrated in Fig. 1. Out of 590 eligible participants, 567 (96.1%) had a SARS-CoV-2 antibody test. The mean participant age was 39.4 years (±12.0); 69.1% (n = 392) were female, 30.5% (n = 173) male, and 0.4% (n = 2) of other sex. A total of (n = 311) 79.3% of female participants had a positive test result, compared with 73.4% (n = 127) of male participants (p = 0.120).

Frequency of loss of sense of smell and taste in the study population
Among the 590 participants who completed questionnaires, 90.0% (n = 531) reported loss of their sense of smell. This was described as complete smell loss by 69.9% (n = 371) and as a partial smell loss by 30.1% (n = 160). No smell loss was reported in 10.0% (n = 59). A total of 89.8% (n = 530) of participants reported loss of their sense of taste. This was described as complete by 47.4% (n = 251) and as partial by 52.6% (n = 279). No taste loss was reported in 10.1% (n = 60) of participants. Combined loss of smell and taste was reported by 80.3% (n = 472).

Seroprevalence of SARS-CoV-2 antibodies in people with acute loss of their sense of smell and/or taste
Of 590 eligible participants, 567 participants (96.1%) underwent SARS-CoV-2 testing. A total of 77.4% (n = 430) had a positive SARS-CoV-2 result (IgG n = 303, IgG and IgM n = 122, and IgM n = 14). One further participant was included with a positive PCR result (77.8% n = 440 positive). Participants with and without SARS-CoV-2 antibodies were comparable in terms of age, sex, ethnicity, smoking status, and frequency of other reported symptoms (Table 2). Importantly, 52.1% (n = 229) of the participants with SARS-CoV-2 antibodies had no history of cough, and 39.8% (n = 175) had neither a fever nor a cough.

Frequency of loss of smell and taste in participants with and without SARS-CoV-2 antibodies
Among the participants who underwent SARS-CoV-2 antibody testing (n = 567), 89.9% (n = 510) reported loss of their sense of smell and 89.7% (n = 509) taste loss. The frequency of reported loss of smell and taste in participants with and without SARS-CoV-2 antibodies are
**Invitation to participate**

Text message invitations sent to 33,650 patients registered with 4 primary care centres in London

**Registration**

23 April — 14 May 2020  
**n = 695**

(10 removed for failed or duplicate registration)  
**n = 685**

**Demographics collected  
**n=685**

- **Age**  
  39.5 years  
  (± 12.3)

- **Sex**
  - Female: 67.5% (n=462)
  - Male: 32.2% (n=221)
  - Other: 0.3% (n=2)

- **Ethnicity**
  - White: 78.1% (n=535)
  - Asian: 5.8% (n=40)
  - Black: 3.4% (n=23)
  - Mixed: 6.7% (n=46)
  - Other: 4.5% (n=31)
  - Not disclosed: 1.5% (n=10)

**Questionnaire completion**

23 April — 15 May 2020  
**n = 650**

60 excluded as ineligible (9.2%)  
**n = 590**

**Testing**

24 April — 22 May 2020  
96.1% tested  
**n = 567**

**Not tested, n=23 (3.9%)**

- 1 withdrawal
- 22 lost to contact

---

Fig 1. Participant flowchart illustrating participant flow through the recruitment process. Participant flowchart illustrating the participant flow through the recruitment process, from test invitations, through eligibility screening, questionnaire completion and testing [13]. Figures presented as % with total number (n). Age presented as mean age in years with standard deviation.

https://doi.org/10.1371/journal.pmed.1003898.g001
Table 2. Study group demographics by SARS-CoV-2 antibody test result.

<table>
<thead>
<tr>
<th>Demographics</th>
<th>SARS-CoV-2 antibody positive (n = 448)</th>
<th>SARS-CoV-2 antibody negative (n = 127)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex (%, with data)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>70.7% (n = 311)</td>
<td>63.8% (n = 81)</td>
<td>0.223</td>
</tr>
<tr>
<td>Male</td>
<td>28.9% (n = 127)</td>
<td>36.2% (n = 46)</td>
<td></td>
</tr>
<tr>
<td>Other</td>
<td>0.3% (n = 2)</td>
<td>0.0% (n = 0)</td>
<td></td>
</tr>
<tr>
<td>Age (mean, SD, years)</td>
<td>39.1 (±8.9)</td>
<td>40.7 (±6.8)</td>
<td>0.250</td>
</tr>
<tr>
<td>Ethnicity (no, % with data)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>White</td>
<td>79.5% (n = 350)</td>
<td>78.7% (n = 100)</td>
<td>0.754</td>
</tr>
<tr>
<td>Asian</td>
<td>6.4% (n = 28)</td>
<td>5.5% (n = 7)</td>
<td></td>
</tr>
<tr>
<td>Black</td>
<td>2.7% (n = 12)</td>
<td>1.6% (n = 2)</td>
<td></td>
</tr>
<tr>
<td>Mixed</td>
<td>5.9% (n = 26)</td>
<td>7.1% (n = 9)</td>
<td></td>
</tr>
<tr>
<td>Other</td>
<td>4.8% (n = 21)</td>
<td>4.7% (n = 6)</td>
<td></td>
</tr>
<tr>
<td>Not disclosed</td>
<td>0.7% (n = 3)</td>
<td>7.4% (n = 3)</td>
<td></td>
</tr>
<tr>
<td>Smoking (% with data)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Current smokers</td>
<td>8.6% (n = 37)</td>
<td>12.6% (n = 16)</td>
<td>0.313</td>
</tr>
<tr>
<td>Non/Ex-smokers</td>
<td>91.6% (n = 403)</td>
<td>87.4% (n = 111)</td>
<td></td>
</tr>
<tr>
<td>Other symptoms (% with data)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cough</td>
<td>47.7% (n = 210)</td>
<td>48.0% (n = 61)</td>
<td>0.952</td>
</tr>
<tr>
<td>Fever</td>
<td>37.7% (n = 167)</td>
<td>25.2% (n = 32)</td>
<td>0.089</td>
</tr>
<tr>
<td>Shortness of breath</td>
<td>37.1% (n = 163)</td>
<td>33.9% (n = 43)</td>
<td>0.351</td>
</tr>
<tr>
<td>Headache</td>
<td>64.3% (n = 283)</td>
<td>63.0% (n = 80)</td>
<td>0.784</td>
</tr>
<tr>
<td>Sore throat</td>
<td>40.9% (n = 180)</td>
<td>52.0% (n = 66)</td>
<td>0.027</td>
</tr>
<tr>
<td>Hoarse voice</td>
<td>12.3% (n = 76)</td>
<td>21.3% (n = 27)</td>
<td>0.305</td>
</tr>
<tr>
<td>Chest pain and/or tightness</td>
<td>37.7% (n = 166)</td>
<td>31.5% (n = 40)</td>
<td>0.198</td>
</tr>
<tr>
<td>Abdominal pain</td>
<td>15.0% (n = 66)</td>
<td>24.4% (n = 31)</td>
<td>0.013</td>
</tr>
<tr>
<td>Diarrhea</td>
<td>25.6% (n = 117)</td>
<td>34.7% (n = 44)</td>
<td>0.076</td>
</tr>
<tr>
<td>Vomiting</td>
<td>3.6% (n = 16)</td>
<td>1.6% (n = 2)</td>
<td>0.343</td>
</tr>
<tr>
<td>Confusion, disorientation and/or drowsiness</td>
<td>32.3% (n = 142)</td>
<td>33.9% (n = 43)</td>
<td>0.737</td>
</tr>
<tr>
<td>Muscle and/or joint pain</td>
<td>66.2% (n = 205)</td>
<td>52.0% (n = 66)</td>
<td>0.096</td>
</tr>
<tr>
<td>Loss of appetite</td>
<td>57.3% (n = 252)</td>
<td>56.0% (n = 71)</td>
<td>0.884</td>
</tr>
</tbody>
</table>

Table comparing age, sex, ethnicity, smoking status as well as additional reported symptoms between participants with positive and negative SARS-CoV-2 antibodies. No significant differences were detected between the two groups, in any variable reported in this table. The significance level for other symptoms was adjusted for multiple comparisons to p = 0.004 through a Bonferroni correction.

SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; SD, standard deviation.

https://doi.org/10.1371/journal.pmed.1903358.kd0

Loss of smell predicts positive SARS-CoV-2 antibody status in a community-based population with an acute loss of their sense of smell and/or taste

Logistic regression was used to explore the relative importance of loss of smell and loss of taste, alone and in combination, as symptoms of COVID-19 infection, assessed by the presence of presented in Fig 2 and Table 3. A total of 80.4% of participants with smell loss and 86.0% (n = 307) of those with complete smell loss had a positive test result. A total of 77.8% of participants with taste loss and 86.0% of those with complete taste loss (n = 209) had a positive test result. Extracts from questionnaire responses from participants with positive SARS-CoV-2 antibodies describing their loss of smell and taste can be seen in Table 4.
SARS-CoV-2 antibodies in our study population with acute loss of smell and taste. Isolated loss of smell and combined loss in the sense of smell and taste were compared with an isolated loss of taste (Table S3).

Participants with loss of smell alone were nearly 3 times more likely than participants with isolated taste loss to have SARS-CoV-2 antibodies (OR 2.86, 95% CI 1.37–6.36; p < 0.001), and participants with combined loss of smell and taste were 4 times more likely to have SARS-CoV-2 antibodies (OR 3.98, 95% CI 2.24–7.08; p < 0.001). These findings remained unchanged after adjusting for sex, age, ethnicity, and smoking status.

Discussion

In this community-based cohort study, undertaken during the peak of the COVID-19 outbreak in London, the seroprevalence of SARS-CoV-2 antibodies in participants with new onset loss of sense of smell and/or taste, was 77.6%. A total of 39.8% (n = 175) of the study
participants reported neither cough nor fever. In our study cohort, loss of smell was more prevalent in participants with SARS-CoV-2 antibodies compared with those without antibodies (93.4% versus 78.7%, \( p < 0.001 \)), whereas taste loss was equally prevalent (90.2% versus 89.0%, \( p = 0.738 \)). Furthermore, participants with acute smell loss were 3 times more to be seropositive for SARS-CoV-2 (OR 2.86; 95% CI 1.27–6.36; \( p < 0.001 \)) compared with those with taste loss.

Loss of smell and taste were evaluated as separate symptoms, which enabled a direct comparison of their relationship to SARS-CoV-2 antibodies. Among participants, loss of sense of smell and taste were evaluated as separate symptoms, which enabled a direct comparison of their relationship to SARS-CoV-2 antibodies.
Table 5. Logistic regression exploring the seroprevalence of SARS-CoV-2 antibodies in people with loss in the sense of smell in isolation, loss in the sense of taste in isolation, and a loss both in the sense of smell and taste in combination.

<table>
<thead>
<tr>
<th>Loss in the sense of taste only (baseline)</th>
<th>Odds ratio (95% CI) (unadjusted)</th>
<th>p-value</th>
<th>Odds ratio (95% CI) (adjusted)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Loss in the sense of smell only</td>
<td>2.86 (95% CI 1.27-6.36)</td>
<td>&lt;0.001</td>
<td>2.72 (95% CI 1.21-6.14)</td>
<td>0.016</td>
</tr>
<tr>
<td>Combined loss in the sense of smell and</td>
<td>3.98 (95% CI 2.24-7.08)</td>
<td>&lt;0.001</td>
<td>4.11 (95% CI 2.29-7.37)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>taste</td>
<td>Constant</td>
<td>0.789</td>
<td>Constant</td>
<td>2.91 (0.75-11.35)</td>
</tr>
</tbody>
</table>

1For sex, age, ethnicity, and smoking status.

CI, confidence interval; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

smell, but not taste, was significantly more prevalent in participants with SARS-CoV-2 antibodies, compared with those without. Moreover, within our study cohort with smell and taste loss, participants with loss of smell alone were 3 times more likely than participants with loss of taste alone to have SARS-CoV-2 antibodies. Participants who reported both a loss of smell and taste were 4 times more likely to have SARS-CoV-2 antibodies compared with participants with isolated taste loss. These findings suggest that a loss of smell is a highly specific symptom of COVID-19, in contrast to a loss of taste, despite their comparable frequency. Sense of taste and smell are interlinked, with retronasal olfaction being a major component of taste (flavor); thus, it is plausible that the loss of taste reported by participants who also report loss of smell reflects impaired retronasal olfaction and hence, represents loss of flavor perception, as a consequence of smell loss. Interestingly, 6.6% of participants with SARS-CoV-2 antibodies reported an isolated loss in their sense of taste, in the absence of smell loss, suggesting the presence of a rarer, alternative pathophysiological mechanism targeting gustatory function in isolation. Lingual mucosal epithelium ACE2 could provide a plausible explanation [19].

Globally, as populations are released from lockdown, early identification by the public of COVID-19 symptoms and rapid self-isolation and testing will be of vital importance to limit disease spread. Currently, as highlighted in Table 1, a large number of countries are not advising that loss of smell and/or taste are COVID-19 symptoms. This could have potentially devastating consequences. Importantly, 40% of our seropositive cohort reported neither fever nor cough. Similarly, a recent UK-based survey reported that cough and/or fever were only present in 31% of people with COVID-19 [3]. Counterintuitively, people with minor symptoms, such as isolated smell loss, who remain systemically well pose the highest public health threat.

SARS-CoV-2 specific antibody testing performed via telemedicine consultation enabled us to confirm participants’ reported symptomatology and verify their identity and test results, which adds to the quality of our data. Although viral nucleic acid detection using real-time PCR remains the gold standard for diagnosis, there are several limitations to this method, including a high processing time, labor intensity, and a high false negative rate [20–22]. Furthermore, there is a narrow window to perform testing before the virus becomes undetectable by PCR [23]. In contrast, seroconversion to IgM and IgG antibodies occurs as early as day 4 [24]. The Center for Disease Control and Prevention recommends antibody testing for diagnostic purposes using high specificity kits to minimize false positives [23]. Given the very high prevalence in our study cohort and the specificity of our chosen test, we could expect a high positive predictive value [23]. The sensitivity of our antibody test is 98.8%. These data were generated in people who were PCR positive, suggesting a very high seroconversion rate.
However, evidence with regard to the rate of patients with confirmed COVID-19, who seroconvert to generate an antibody response remains limited. Current data suggest seroconversion rates of up to 100% in hospital patients, which may be related to disease severity but also time from symptom onset [20,25,26]. Although a recent study demonstrated a 99.4% seroconversion rate in hospital staff following a mild COVID-19 illness, in which 47.5% of the study population reported anosmia [27], no data are available yet reporting the rate of seroconversion in confirmed COVID-19 patients with anosmia.

Limitations

The main limitation of our study is the lack of a general population control group without loss of smell and/or taste. The study only recruited participants who reported acute smell and/or taste loss. Although this enabled us to study this presentation and its relevance to COVID-19, this also presents a degree of selection bias, hence, our findings refer to a population subset with new acute loss of smell and/or taste. In addition, although we used a questionnaire to assess COVID-19 symptoms and subsequently validated participants' responses during the telemedicine interview, we did not undertake any objective assessments of smell or taste. However, from a COVID-19 disease containment perspective, the general public are unlikely to have rapid access to formal objective smell/taste testing. Moreover, our data show a high seroprevalence of SARS-CoV-2 antibodies in people who recognized a loss of their sense of smell. Furthermore, as our study relied on antibody testing via telemedicine, we were unable to account for COVID-19 patients who may not have seroconverted to develop IgG or IgM antibodies, which may lead to our findings underestimating the prevalence of COVID-19 in this population. However, our findings suggest that a key public health message is that people who notice a loss in their ability to smell everyday household odors such as garlic, onions, coffee, and perfumes should self-isolate and seek PCR testing.

The majority of our participants were female; this finding may reflect previous findings that females have a higher frequency of loss of smell and/or taste with COVID-19 than males [28,29]. We found no differences in sex, but this may reflect the lower number of males recruited.

Conclusions

In a community-based population, the vast majority of participants with new onset loss of smell were seropositive for SARS-CoV-2 antibodies. Acute loss of sense of smell needs to be considered globally as a criterion for self-isolation, testing, and contact tracing in order to contain the spread of COVID-19.

Supporting information

S1 STROBE checklist. STROBE, Strengthening the Reporting of Observational Studies in Epidemiology.

(DOCX)

S1 Text. Questionnaires used to capture demographics (part 1) and symptoms in study participants (part 2).

(DOCX)

S1 Data. Study data set.

(XLSX)
Acknowledgments

We thank Jed Wingrove for designing the participant instruction leaflet and Charalampos Markakis for his help in the organization and running of the study. We also thank the Hampstead GP Practice, The Northern Medical Centre, the James Wigg Practice, and the Queen’s Crescent Practice for their collaboration in sending out the invitation text messages to their registered patients.

Author Contributions

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Funding acquisition: Rachel L. Batterham.

Investigation: Janine Makaronidis, Jessica Mok, Nyalodzi Balogun, Cormac G. Magee, Alisia Camenolla, Rachel L. Batterham.

Methodology: Cormac G. Magee, Alisia Camenolla, Rachel L. Batterham.

Project administration: Alisia Camenolla.

Resources: Rachael L. Batterham.

Validation: Janine Makaronidis.

Writing – original draft: Janine Makaronidis, Jessica Mok, Nyalodzi Balogun, Cormac G. Magee, Runama Z. Omar, Alisia Camenolla, Rachel L. Batterham.

Writing – review & editing: Janine Makaronidis, Jessica Mok, Nyalodzi Balogun, Cormac G. Magee, Runama Z. Omar, Alisia Camenolla, Rachel L. Batterham.

References


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Distorted chemosensory perception and female sex associate with persistent smell and/or taste loss in people with SARS-CoV-2 antibodies: a community based cohort study investigating clinical course and resolution of acute smell and/or taste loss in people with and without SARS-CoV-2 antibodies in London, UK

Janine Makaronidis, Chloe Firman, Cormac G. Magee, Jessica Moir, Nyaladé Balogun, Matt Lechner, Alisia Carnemola, and Rachel L. Batterham

Abstract

Background: Loss of smell and/or taste are cardinal symptoms of COVID-19. 'Long-COVID', persistence of symptoms, affects around one fifth of people. However, data regarding the clinical resolution of loss of smell and/or taste are lacking. In this study we assess smell and taste loss resolution at 4-6 week follow-up, aim to identify risk factors for persistent smell loss and describe smell loss as a feature of long-COVID in a community cohort in London with known SARS-CoV-2 IgG/IgM antibody status. We also compare subjective and objective smell assessments in a subset of participants.

Methods: Four hundred sixty-seven participants with acute loss of smell and/or taste who had undergone SARS-CoV-2 IgG/IgM antibody testing 4–6 weeks earlier completed a follow-up questionnaire about resolution of their symptoms. A subsample of 50 participants completed an objective olfactory test and results were compared to subjective smell evaluations.

(Continued on next page)
Introduction
Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2), which results in coronavirus disease 2019 (COVID-19), emerged in 2019 resulting in a global pandemic with over 85 million cases and 1.8 million deaths reported worldwide [1]. The association between COVID-19 and smell and taste loss was established in March 2020 and has since been identified among the most specific symptoms of COVID-19, with implications for case identification, isolation and tracing [2, 3]. We previously reported seroprevalence of SARS-CoV-2 IgG/IgM antibodies of 78% in a community cohort in London, UK who developed an acute loss of their sense of smell and/or taste during the peak of the first local wave of the pandemic [4]. We also showed that seropositivity for SARS-CoV-2 antibodies was three times more likely for people with smell loss compared to taste loss.

With the ongoing spread of SARS-CoV-2 globally, COVID-19 and its complications are continuing to affect millions globally. Despite the development of vaccinations resulting in optimism that the pandemic will be contained, healthcare providers worldwide will continue to face the challenge of an unprecedented incidence of COVID-19 related morbidity. It is now evident that symptoms can persist beyond resolution of the acute systemic viral infection and cause a chronic condition, termed ‘long-COVID’, in up to a quarter of cases [5]. ‘Long covid’ (>4 weeks) as well as ‘post-acute covid’ (>3 weeks) and ‘chronic covid’ (>12 weeks) have been used to describe ongoing symptoms of the disease [6, 7]. Symptoms persisting longer than 5 weeks are estimated to occur in 1 in 5 and symptoms lasting longer than 12 weeks in 1 in 10 people, according to data published by the UK Office for National Statistics (ONS) [8].

Identifying the people most at risk of long-term symptoms will be key to guide monitoring in order to deliver follow-up care and therapeutic interventions to patients with long COVID-19 disease.

Despite the recognition of loss of smell and/or taste as key symptoms of COVID-19 there remains a paucity of data within the literature regarding the clinical course, recovery rates and demographic risk factors for long-lasting symptoms. Post-viral smell loss from other respiratory pathogens is typically a short-lived phenomenon and recovery usually coincides with resolution of other viral symptoms [9, 10]. In contrast, current data from COVID-19 patients, suggest a wide range of recovery times, from a few days to several months [11–13]. This observation raises concerns of unresolved smell loss as a manifestation of long-COVID. Therefore, identifying risk factors for persistent smell loss will be key to guide therapeutic interventions, such as olfactory rehabilitation and use of corticosteroids, once these are widely available [14, 15]. Furthermore, given the negative impact of these symptoms on quality of life and safety, research on prognostic information is warranted to inform patients, their carers, as well as treatment algorithms [16]. In this follow-up study, we aim to describe and compare the temporal resolution patterns of loss of smell and/or taste in a community cohort with acute smell and/or taste loss with and without SARS-CoV-2 antibodies. This study will focus on smell loss resolution and aim to identify risk factors for persistent smell loss, as well as describe smell loss as a feature of long-COVID. Finally, to address the original study’s limitations of reliance on self-reported symptoms, we will correlate self-reported smell function with objective olfactory testing.
Methods
The study was conducted in London, UK. Participants were recruited between 23 April and 14 May 2020, during the local first wave of the pandemic, prior to recognition of smell and taste loss as symptoms of COVID-19. Primary care centres sent out text messages to invite people with an acute loss of their sense of smell and/or taste to participate. Participants were recruited via an online platform, as previously described [4].

Inclusion criteria were age of 18 years and above, being proficient in written and spoken English and having access to a device that can perform video calls. Participants who experienced a loss of the sense of smell or taste with a duration greater than 1 month were excluded [4]. Participation was voluntary and written informed consent was obtained electronically. Participants were enrolled through consecutive recruitment of eligible participants, who completed an online questionnaire (see Additional File 1). The baseline questionnaire included questions on participants' demographics and their symptoms [4].

SARS-CoV-2 immunoglobulin G (IgG) / immunoglobulin M (IgM) antibody testing was carried out via a telemedicine consultation as previously described [4], scheduled taking into consideration the participants' date of symptom onset. The test used was a lateral flow immunosay detecting IgM and IgG antibodies to SARS-CoV-2 (Wuhan UNScience Biotechnology Co., Ltd. COVID-19 Antibody IgM/IgG) and has a relative sensitivity of 98.8% (95% CI 97.3–99.6%) and a relative specificity of 98.0% (95% CI 97.15–98.7%) [17]. A link to a follow-up questionnaire was sent to participants' registered email addresses 4 weeks after they completed their original questionnaire. The follow-up questionnaire (see Additional File 1) contained questions about resolution of their smell and taste loss, as well as resolution of the other symptoms of COVID-19 and admission to hospital. Reminders were sent to participants who did not complete the questionnaire at 72 h and 7 days and the platform remained open for questionnaire completion for a further 4 weeks (22 May to 20 July 2020).

In order to correlate participants' questionnaire responses about their loss of smell with objective olfactory testing, a subsample of 50 participants were recruited for objective smell testing. Participants received a separate information sheet and informed consent was obtained electronically using a separate consent form. The University of Pennsylvania Smell Identification Test (UPSIT), a 40-item smell test which is validated to be self-administered, was used [18]. UPSIT kits were sent to recruited participants together with instructions on completing the test. In view of the time elapsed between the completion of the follow-up questionnaire and the UPSIT testing, prior to testing, participants were asked how they subjectively perceived their smell function. Photographs of the booklet were obtained and the tests were scored by a healthcare professional, who then explained the results to participants. Participants' subjective smell function was correlated with their UPSIT results.

Ethical approval for this study was issued by the National Health Service Queen's Square Research Ethics Committee (IRAS Project ID 282668, Clinicaltrials.gov: NCT04377815). The study was conducted in line with the declaration of Helsinki and Good Clinical Practice.

Statistical analysis
The recruitment target for this study was set at 500 participants. A sample size calculation yielded a sample size of 385, which including a 13% attrition rate was initially increased to 453 participants and then increased to 500 to allow for larger attrition [4]. The recruitment target was exceeded to increase accuracy.

Data analysis was performed using GraphPad Prism version 8 (https://www.graphpad.com/scientific-software/prism/) and SPSS version 26 (https://www.ibm.com/uk-en/products/spss-statistics). Descriptive statistics were performed; means (plus standard deviation [SD]) were calculated for continuous variables and numbers (n, with percentages) for categorical variables. Categorical data were analysed using chi-squared tests. The significance level for multiple comparisons was adjusted using a Bonferroni correction. Parametric and non-parametric tests were used as appropriate to analyse continuous data. Logistic regression analysis was performed to estimate the association between smell loss resolution and additional factors including participant's age, sex, ethnicity, smoking status and smell loss pattern. A Spearman Rank correlation analysis was performed to investigate the association between participants' perceived smell function and an objective assessment of their olfactory function.

Results
Study population
A total of 467 out of the 569 participants who enrolled and underwent a SARS-CoV-2 antibody test completed the follow-up questionnaire, yielding a follow-up rate of 82.1%. The demographics of the entire study cohort can be seen in Additional File 2. Out of the cohort of 467 who completed the follow-up questionnaire, participants with positive and negative SARS-CoV-2 antibodies were comparable in terms of age, gender, smoking status and ethnicity (Table 1). Admission to hospital was reported by 1.6% (n = 7) of participants in the antibody positive group vs. 3.1% (n = 4) in the antibody negative group (p = 0.097).
Table 1: Demographics of participants followed up after 4–6 weeks with positive and negative SARS-CoV-2 antibodies

<table>
<thead>
<tr>
<th>Demographics</th>
<th>SARS-CoV-2 IgG/IgM positive (n = 381)</th>
<th>SARS-CoV-2 IgG/IgM negative (n = 86)</th>
<th>p-value (0.05)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>70.9% (n = 270)</td>
<td>66.3% (n = 57)</td>
<td>0.382</td>
</tr>
<tr>
<td>Male</td>
<td>28.1% (n = 110)</td>
<td>33.7% (n = 29)</td>
<td></td>
</tr>
<tr>
<td>Other</td>
<td>0.3% (n = 1)</td>
<td>0</td>
<td>0.450</td>
</tr>
<tr>
<td>Age (years)</td>
<td>39.57 ± 12.12</td>
<td>40.25 ± 12.31</td>
<td>0.689</td>
</tr>
<tr>
<td>Ethnicity *</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>White</td>
<td>83.7% (n = 319)</td>
<td>81.4% (n = 70)</td>
<td>0.600</td>
</tr>
<tr>
<td>Mixed/Multiple Ethnicities</td>
<td>5.5% (n = 21)</td>
<td>4.7% (n = 4)</td>
<td>0.749</td>
</tr>
<tr>
<td>Asian/Asian British</td>
<td>5% (n = 19)</td>
<td>4.7% (n = 4)</td>
<td>0.897</td>
</tr>
<tr>
<td>Black/African/Caribbean/Black British</td>
<td>1.6% (n = 6)</td>
<td>2.3% (n = 2)</td>
<td>0.628</td>
</tr>
<tr>
<td>‘Other’</td>
<td>3.7% (n = 14)</td>
<td>3.3% (n = 3)</td>
<td>0.933</td>
</tr>
<tr>
<td>Smoking status</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Current/ Ex-smoker</td>
<td>42% (n = 160)</td>
<td>44.2% (n = 38)</td>
<td>0.710</td>
</tr>
<tr>
<td>Never smoked</td>
<td>58% (n = 221)</td>
<td>55.8% (n = 48)</td>
<td></td>
</tr>
</tbody>
</table>

Figures presented as % with total number (n). SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

*Participants opted not to disclose their ethnicity

Smell and taste loss and resolution at 4–6 weeks follow-up
The frequency of reported smell and/or taste loss at baseline in participants who completed the follow-up questionnaire can be seen in Table 2.

Out of 467 patients followed up at 4–6 weeks, 57.7% (n = 264) of participants with positive SARS-CoV-2 antibodies reported full resolution of their smell loss, compared to 72.1% (n = 49) of participants with a negative antibody test (p = 0.027). Out of the participants with positive SARS-CoV-2 antibodies, 38.4% (n = 150) reported partial and 3.5% (n = 14) reported no resolution of their smell loss at the time of follow-up. Out of participants with negative SARS-CoV-2 antibodies 25.0% (n = 17) reported partial and 2.9% (n = 2) no resolution of their smell loss.

Full resolution of taste loss was reported by 66.2% (n = 227) of participants with SARS-CoV-2 antibodies and 80.3% (n = 61) of participants with negative SARS-CoV-2 antibodies (p = 0.017). Out of participants with positive SARS-CoV-2 antibodies, 31.2% (n = 107) reported partial and 2.6% (n = 9) no resolution of their taste loss at the time of follow up. Out of participants with negative SARS-CoV-2 antibodies, 19.7% (n = 15)

Table 2: Loss of smell and/or taste in SARS-CoV-2 IgG/IgM positive and negative participants

<table>
<thead>
<tr>
<th>Sense of smell</th>
<th>SARS-CoV-2 IgG/IgM positive (n = 381)</th>
<th>SARS-CoV-2 IgG/IgM negative (n = 86)</th>
<th>p-value (0.05)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Loss of sense of smell (complete and partial)</td>
<td>93.7% (n = 350)</td>
<td>79.1% (n = 66)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Partial loss of smell</td>
<td>23.7% (n = 90)</td>
<td>47.1% (n = 32)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Complete loss of smell</td>
<td>73.4% (n = 200)</td>
<td>57.9% (n = 30)</td>
<td></td>
</tr>
<tr>
<td>Parosmia (distorted sense of smell)</td>
<td>29.7% (n = 113)</td>
<td>22.1% (n = 19)</td>
<td>0.159</td>
</tr>
<tr>
<td>Sense of taste</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Loss of sense of taste (complete and partial)</td>
<td>89.8% (n = 342)</td>
<td>85.4% (n = 70)</td>
<td>0.304</td>
</tr>
<tr>
<td>Partial loss of taste</td>
<td>54.1% (n = 185)</td>
<td>68.9% (n = 53)</td>
<td>0.013</td>
</tr>
<tr>
<td>Complete loss of taste</td>
<td>45.9% (n = 157)</td>
<td>31.1% (n = 23)</td>
<td></td>
</tr>
<tr>
<td>Dysegeusia (distorted sense of taste)</td>
<td>43.0% (n = 167)</td>
<td>30.6% (n = 31)</td>
<td>0.187</td>
</tr>
<tr>
<td>Experience of taste without eating/drinking</td>
<td>19.9% (n = 76)</td>
<td>25.3% (n = 20)</td>
<td>0.492</td>
</tr>
<tr>
<td>Only loss of smell</td>
<td>10% (n = 36)</td>
<td>11.8% (n = 10)</td>
<td>0.699</td>
</tr>
<tr>
<td>Only loss of taste</td>
<td>6.3% (n = 24)</td>
<td>20.9% (n = 18)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Loss of sense of smell and taste (partial and complete)</td>
<td>83.7% (n = 319)</td>
<td>67.4% (n = 50)</td>
<td>&lt; 0.001</td>
</tr>
</tbody>
</table>
reported partial resolution of their taste loss (0 participants reported ‘no resolution’ of taste loss). Out of participants with positive SARS-CoV-2 antibodies, only 24 experienced a loss of their sense of taste in the absence of a loss of smell. The demographics were comparable to those of the entire SARS-CoV-2 positive cohort, with a mean age of 38.4 ± 14.21 years and 70.8% (n = 17) of participants of female sex.

For subsequent analyses participants with partial and no resolution were grouped together in order to enable comparison between participants who achieved full resolution versus those who had ongoing smell and/or taste impairment at the time of follow-up. Table 3 shows results regarding resolution vs. no resolution of smell loss, taste loss and combined smell/taste loss in participants with positive and negative SARS-CoV-2 antibodies. A higher percentage of participants without SARS-CoV-2 antibodies fully recovered their sense of smell (72.1% vs 57.7%, p = 0.027), their sense of taste (60.3% vs 66.2%, p = 0.017) and both their senses of smell and taste (79.6% vs 64.0%, p = 0.026).

In participants with SARS-CoV-2 antibodies who reported full resolution of their smell loss, a full recovery of the sense of smell was reported to have occurred within 1 week in 11.7%, within 1–2 weeks in 36%, within 2–4 weeks in 26.5% and within >4 weeks in 35.8%.

The effects of smell loss pattern and presence of parosmia on recovery of the sense of smell in participants with SARS-CoV-2 IgG/IgM antibodies

The effect of complete vs. partial smell loss and the presence of parosmia on smell recovery in participants with positive SARS-CoV-2 antibodies was investigated. In participants who experienced complete loss of their sense of smell, full sense of smell recovery was reported by 54.5% compared to 67.4% in participants who reported a partial loss of their sense of smell (54.5% vs 67.4%, p = 0.032).

Out of participants who reported parosmia at the time of their smell loss, full recovery was reported by 41.4% compared to 65% in participants with smell loss who did not experience parosmia (41.4% vs 65%, p < 0.001).

The effects of taste loss pattern and presence of dysgeusia on recovery of the sense of taste in participants with SARS-CoV-2 IgG/IgM antibodies

The effect of complete vs. partial taste loss and the presence of dysgeusia on smell recovery in participants with positive SARS-CoV-2 antibodies was investigated. There was no significant difference in the reported rates of recovery of taste loss in participant who reported complete vs. partial loss of their sense of taste (64.7% vs 67.9%, p = 0.525).

Out of participants who experienced dysgeusia at time of their loss of taste, a significantly lower proportion reported full resolution of their taste loss, compared to participants who did not experience dysgeusia (60.7% vs 71.4%, p = 0.036).

Participants who experienced taste sensations in the absence of eating or drinking reported lower resolution rates compared to participants who did not (51.2% vs 71.0%, p = 0.001).

The effect of sex and age on the recovery of the sense of smell and taste in participants with SARS-CoV-2 IgG/IgM antibodies

Full recovery of sense of smell was more prevalent among males compared to females (72.8% in males vs. 51.4% in females, p < 0.001). Similarly, full taste loss resolution was more common in males vs. females (80.8% vs 60.1%, p < 0.001) as well as full resolution of combined smell/taste loss (69.6% vs 41.1%, p < 0.001); Table 4.

The effect of age on resolution of smell and taste loss was evaluated. Mean age of male and female participants was comparable for participants who experienced full resolution of the loss in their sense of smell (40.4 ± 13.2 years in males vs. 38.1 ± 11.3 in females, p = 0.333), their sense of taste (40.3 ± 13.4 vs 37.1 ± 10.7 p = 0.153) and combined loss of smell and taste (40.0 ± 12.6 vs 37.1 ± 13.4 years in males vs. 38.1 ± 11.3 in females, p = 0.333).

Table 3 Smell and/or taste loss resolution in SARS-CoV-2 IgG/IgM positive and negative participants

<table>
<thead>
<tr>
<th>Pattern of resolution</th>
<th>SARS-CoV-2 IgG/IgM positive</th>
<th>SARS-CoV-2 IgG/IgM negative</th>
<th>p-Value (0.05)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Smell loss</td>
<td>Total (n = 357)</td>
<td>Total (n = 61)</td>
<td></td>
</tr>
<tr>
<td>No/partial resolution</td>
<td>42.3% (n = 151)</td>
<td>27.9% (n = 19)</td>
<td>0.027</td>
</tr>
<tr>
<td>Full resolution</td>
<td>57.7% (n = 206)</td>
<td>72.1% (n = 42)</td>
<td></td>
</tr>
<tr>
<td>Taste loss</td>
<td>Total (n = 343)</td>
<td>Total (n = 76)</td>
<td></td>
</tr>
<tr>
<td>No/partial resolution</td>
<td>33.8% (n = 116)</td>
<td>19.7% (n = 15)</td>
<td>0.017</td>
</tr>
<tr>
<td>Full resolution</td>
<td>66.2% (n = 227)</td>
<td>80.3% (n = 61)</td>
<td></td>
</tr>
<tr>
<td>Combined smell and taste loss</td>
<td>Total (n = 261)</td>
<td>Total (n = 54)</td>
<td></td>
</tr>
<tr>
<td>No/partial resolution</td>
<td>36.0% (n = 94)</td>
<td>20.4% (n = 11)</td>
<td>0.026</td>
</tr>
<tr>
<td>Full resolution</td>
<td>64.0% (n = 167)</td>
<td>79.6% (n = 43)</td>
<td></td>
</tr>
</tbody>
</table>
Table 4 Resolution of loss of smell, loss of taste and combined loss of smell and taste in female vs. male participants with SARS-CoV-2 IgG/IgM antibodies

<table>
<thead>
<tr>
<th>Resolution</th>
<th>Female (N = 276)</th>
<th>Male (N = 110)</th>
<th>p-value (0.05)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Small loss resolution</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Full resolution</td>
<td>51.4% (n = 30)</td>
<td>72.8% (n = 75)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>No/partial resolution</td>
<td>48.6% (n = 123)</td>
<td>27.1% (n = 28)</td>
<td></td>
</tr>
<tr>
<td>Taste loss resolution</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Full resolution</td>
<td>50.1% (n = 140)</td>
<td>80.0% (n = 83)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>No/partial resolution</td>
<td>49.9% (n = 77)</td>
<td>20.0% (n = 17)</td>
<td></td>
</tr>
<tr>
<td>Combined smell and taste loss</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Full resolution</td>
<td>45.1% (n = 120)</td>
<td>69.5% (n = 74)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>No/partial resolution</td>
<td>54.9% (n = 156)</td>
<td>30.5% (n = 36)</td>
<td></td>
</tr>
</tbody>
</table>

11.0, p = 0.122). In participants with loss of their sense of taste that did not resolve at the time of follow-up, mean age was significantly higher in females compared to males (42.7 ± 12.5 years vs. 37.6 ± 12.6 years, p = 0.030). Mean age was also significantly higher in female participants with unresolved combined loss of smell and taste loss compared to male participants (42.8 ± 12.5 vs. 34.6 ± 10.4, p = 0.001). Mean age of female participants with unresolved smell loss was 41.6 ± 11.7 years compared to 37.4 ± 12.7 years in male participants, however this borderline difference did not reach statistical significance (p = 0.053).

In light of the above findings, we further evaluated the effect of age on smell and/or taste loss resolution in female participants. A significantly higher age was observed in female participants without resolution compared to those with full resolution of the loss of their sense of smell (41.6 ± 11.7 yrs. vs. 38.1 ± 11.3 yrs., p = 0.010), their sense of taste (42.7 ± 12.5 yrs. vs. 37.1 ± 10.7 yrs., p < 0.001) and combined smell and taste (428 ± 12.5 yrs. vs. 37.1 ± 11.0 yrs., p < 0.001).

Predictors of persisting smell loss in a community population with SARS-CoV-2 antibodies and acute loss of their sense of smell

Logistic regression was used to explore the relative importance of participant’s age, sex, ethnicity, smoking status, presence of parosmia and smell loss pattern as risk factors for persistent smell loss at >4 weeks from onset.

Female participants were almost 2.5 times more likely to have ongoing smell loss after 4 weeks compared to participants of male sex (OR 2.46, 95% CI 1.47 to 4.13, p = 0.001). Parosmia was also shown to have a significant association with unresolved smell loss at 4–6 week follow-up (OR 2.47, 95%CI 1.54 to 4.00, p < 0.001), in a model adjusting for the age, ethnicity, patterns of smell loss (complete vs partial) and smoking Table 5.

Persistent smell and/or taste loss as a manifestation of long COVID

At the end of the 4–6 week follow-up period 42.3% (n = 151) of participants with positive SARS-CoV-2 antibodies had ongoing smell loss, 33.8% experienced ongoing taste loss and 36% had ongoing taste and smell loss. We also evaluated the resolution of other symptoms of COVID-19 at the end of the follow-up period in participants positive for SARS-CoV-2 antibodies. Out of 134 participants with unresolved smell loss who reported additional COVID-19 symptoms on their original questionnaire, 29.1% (n = 39) had at least 1 additional unresolved symptom at the time they completed their follow-up questionnaire, compared to 19.9% (n = 35) of participants with full resolution of their smell loss (29.1% vs 19.9%, p = 0.059). The most commonly reported unresolved

Table 5 Logistic regression exploring the association between age, sex, ethnicity, smoking status, presence of parosmia and smell loss pattern (complete vs partial) and no resolution of smell loss at 4 weeks follow up

<table>
<thead>
<tr>
<th>Variable</th>
<th>B</th>
<th>OR</th>
<th>55% CI (lower)</th>
<th>55% CI (upper)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>0.13</td>
<td>1.01</td>
<td>0.94</td>
<td>1.02</td>
<td>0.172</td>
</tr>
<tr>
<td>Ethnicity</td>
<td>0.09</td>
<td>1.10</td>
<td>0.95</td>
<td>1.26</td>
<td>0.002</td>
</tr>
<tr>
<td>Complete anosmia</td>
<td>0.059</td>
<td>1.69</td>
<td>0.988</td>
<td>2.884</td>
<td>0.051</td>
</tr>
<tr>
<td>Parosmia</td>
<td>0.004</td>
<td>2.46</td>
<td>1.539</td>
<td>3.966</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Sex (Female)</td>
<td>0.001</td>
<td>2.46</td>
<td>1.468</td>
<td>4.126</td>
<td>0.001</td>
</tr>
<tr>
<td>Smoking</td>
<td>0.003</td>
<td>1.35</td>
<td>0.604</td>
<td>3.038</td>
<td>0.462</td>
</tr>
</tbody>
</table>
symptoms were shortness of breath, chest pain and muscle/joint pains.

Objective smell testing in a subsample of participants and correlation with perceived smell function

A subsample of 50 participants underwent objective olfactory testing using the UPSIT. 84% were female (n = 42) and 16% (n = 8) male. The mean age was 39.6 ± 13.5 years and mean duration of test date from the onset of symptoms was 21.6 ± 4.7 weeks. 76% (n = 38) of participants had complete loss of their sense of smell at the time of the original questionnaire and 24% (n = 12) partial loss of smell. At the time of the follow-up questionnaire 16% (n = 8) reported their smell loss 'did not resolve', 42% (n = 21) reported their smell loss 'resolved partially' and 42% (n = 21) reported their smell loss 'resolved fully'.

In view of the time elapsed between the completion of the follow-up questionnaire and the UPSIT testing prior to testing, participants were asked how they subjectively perceived their smell function at the time of the UPSIT test. Their answers were grouped into 'No or minimal sense of smell', 'Sense of smell improved but not fully recovered' or 'Sense of smell fully recovered'. The mean UPSIT test score was 29.1 ± 7.5 points. UPSIT testing revealed total anosmia in 5 participants (10%), severe microsmia in 5 (10%), moderate microsmia in 8 (16%), mild microsmia in 6 (12%) and normosmia (normal smell function) in 26 (52%).

Table 6 illustrates a comparison of participants' perceived smell function and their UPSIT test result, by test result category. A Spearman rank correlation analysis found a significant correlation between perceived smell function and UPSIT test result category (r = 0.84 ± 0.71 to 0.50, p < 0.001).

Discussion

We report longitudinal data from a community cohort with a new loss in their sense of smell and/or taste and resolution of these symptoms both in people with positive and negative SARS-CoV-2 IgG/IgM antibodies. Our data come from an entirely community based cohort with a low hospital admission rate where loss of taste and/or smell are the predominant symptoms. 77.9% of our cohort had positive SARS-CoV-2 antibodies and the study had a follow-up completion rate of 82.2%. We report a higher rate of recovery of smell loss (72.1% vs. 57.7%; p = 0.027), taste loss (80.3% vs. 66.2%; p = 0.017) and combined smell and taste loss (79.6% vs. 64%; p = 0.026) in participants who tested negative compared to participants who tested positive for SARS-CoV-2 antibodies. Participants in this study were tested for antibodies following recruitment and completing the baseline questionnaire, taking into consideration their onset of symptoms. Evidence into persistence of these antibodies now suggests that these persist for several months, even in populations with mild disease [19, 20].

Importantly, our study highlights the high percentage of patients with ongoing smell loss (42.3%), ongoing taste loss (33.8%) and combined smell and taste loss (36.0%). The observed smell loss resolution rate of 57.7% in participants with SARS-CoV-2 antibodies within 4–6 weeks in our study is in line with existing literature [12, 21]. Dell’Era et al. similarly reported that in 355 participants with COVID-19, 70% reported either smell loss and/or taste loss during infection [22]. 49.5% of participants reported full resolution of both sense of smell/taste after 14 days since the onset of symptoms, increasing to 62.9% at time of interview (23 days median, range 15–31), with a median recovery time of 10 days. In contrast to our community-based study, their findings come from a hospitalised patient cohort. Resolution rates in the literature currently range from 20 to 92.8% [11, 12, 23]. Discrepancies are likely due to differences in study populations, sample size, location and duration of follow up since onset of symptoms.

Furthermore, we report higher rates of smell loss resolution in participants with partial compared to complete smell loss (67.4% vs. 54.5%, p = 0.032). This is compatible with Kosugi et al. [14] who reported that the full resolution from 'partial loss of smell' in COVID-19 positive patients takes place more frequently than that from 'complete loss of smell'. Supportively, using an objective approach, LaChien et al. also found that higher baseline severity of smell loss, measured by 'Sniffin’ Sticks' was strongly predictive of persistent smell loss [24]. Beltrán-Corbíllini et al. compared smell loss recovery in 70 COVID-19 and 40

<table>
<thead>
<tr>
<th>Table 6</th>
<th>Comparison between UPSIT test result and perceived smell function in a study subgroup of 50 participants</th>
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<tbody>
<tr>
<td>Minimal/no sense of smell (n = 11)</td>
<td>Improved sense of smell, not fully recovered (n = 9)</td>
</tr>
<tr>
<td>Total anosmia</td>
<td>45.5% (5)</td>
</tr>
<tr>
<td>Severe microsmia</td>
<td>45.5% (5)</td>
</tr>
<tr>
<td>Moderate microsmia</td>
<td>90% (1)</td>
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<tr>
<td>Mild microsmia</td>
<td>0%</td>
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<tr>
<td>Normosmia</td>
<td>0%</td>
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<td>Spearman r</td>
<td>0.25</td>
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flu and reported full resolution after 7.4 ± 2.3 days and 16.7% reported partial resolution after 9.1 ± 3.6 days, whereas 62% of influenza participants fully recovered their sense of smell. The fact that participants with SARS-CoV-2 antibodies also had higher rates of complete anosmia, suggests a more severe spectrum of COVID-19 related smell loss compared to post-viral smell loss from other respiratory pathogens.

Interestingly, one of our key findings shows that parosmia was more common in the group of participants with unresolved smell loss and was also a predictor of non-remission in the logistic regression analysis, which is novel in COVID-19. Parosmia has been associated with post-viral smell loss prior to the COVID-19 pandemic [26]. A potential explanation for our finding may be that parosmia has been associated with decreased number and disordered regrowth of olfactory axons into existing neural circuits and a preponderance of immature neurons [27]. Comparatively, Liu et al found that in 153 participants with post-infectious smell loss the presence of parosmia was associated with clinically significant recovery in supra-threshold olfactory function discrimination in patients receiving olfactory training [28]. Parosmia in the context of post-viral smell loss is associated with ongoing smell impairment, and although has been viewed as a sign of recovery, its role as a prognostic marker remains largely unclear. However, our data suggest that parosmia is a marker of poor prognosis in COVID-19. Similarly, our finding show that dysgeusia and experiencing taste sensations in the absence of eating and drinking were associated with lower reported taste loss resolution rates. Together, these findings suggest that distorted chemosensory perception is a risk factor for prolonged smell and/or taste loss and long-COVID.

With regard to full resolution of smell and/or taste loss, a significant sex difference was evident. Females had a lower full resolution rate of smell loss (51.4% vs. 72.8%; p < 0.001), taste loss (60.1% vs. 80.8%; p < 0.001) and combined smell and taste loss (45.1% vs. 69.6%; p < 0.001), compared to males, respectively. These findings are supported by several studies who also report the recovery of sense of smell was longer in females [10, 22]. Although Meini et al. found that the recovery rate did not differ significantly between males and females, they however, the mean recovery time from smell loss or taste loss was significantly longer for females than for males (26 vs. 14 days, p = 0.009), even though the mean age of males was significantly higher than that of females (66 vs. 57 years, p = 0.04) [21].

Notably, in our study, female participants who reported no/partial resolution of smell loss were significantly older (41.6 ± 11.7 years) than female participants who reported full resolution of smell loss (38.1 ± 11.3 years, p = 0.010). The same was also found to be true for taste loss (42.7 ± 12.5 vs. 37.1 ± 10.7, p < 0.001) and combined smell and taste loss (42.8 ± 12.5 vs. 37.1 ± 11.0, p < 0.001). In contrast, Lee et al. reported that young age, particularly the age group of 20–39 years, showed a tendency to be associated with a longer persistence of anosmia [29]. Interestingly, oestradiol has been shown to increase olfactory epithelial cell density and to have a protective role against olfactory function decline [30]. Additionally, in animal models of neurodegenerative diseases oestradiol replacement prevents olfactory dysfunction [31]. This study was not powered to estimate the effect of menopause-driven differences in this subgroup. Nevertheless, a link between prolonged or reduced recovery of smell function and a post-menopausal state appears plausible.

Loss of smell and taste have been demonstrated to persist beyond resolution of the infectious phase of COVID-19 [32]. In light of evidence from the clinical course of smell loss from other viruses, smell loss after COVID-19 could persist for two or more years [33, 34]. Arguably, our study follow-up window still only represents a relatively short-term observation and it is reasonable to predict that a number of participants will have further recovered their sense of smell and taste over additional weeks. Nevertheless, loss of smell and taste undoubtedly constitute a manifestation of long-COVID, which may result in significant psychological morbidity and adversely impact quality of life of the subset of patients with long-term unresolved smell and taste loss [8, 16, 35]. Given the limitations in accuracy and accessibility to testing during particularly the earlier phases of the pandemic, further patient populations may present with olfactory and gustatory impairment and features of long-COVID, indicative of previous COVID-19 infections. Of note, current proposed diagnostic criteria for long-COVID or chronic COVID-19 do not necessitate a positive test at time of symptom onset. The magnitude of the pandemic and the potential for SARS-CoV-2 to cause long-term smell and/or taste loss in relatively small cohorts therefore suggest that the overall prevalence of long-COVID with smell loss within the general population will be considerable [5, 9].

It will be vital to develop a better understanding of the pathophysiology causing smell loss as well as the physiological processes driving smell recovery, in order to facilitate development of effective treatments for smell loss. Angiotensin-converting enzyme-2 receptors (ACE-2) present on olfactory epithelial cells and neuropilin-1 receptors (NRPI), abundant on all olfactory cell lines, are known entry routes for SARS-CoV-2 and damage to these cells have been proposed as potential mechanisms for smell loss [36–38]. Viral entry via NRPI could result
in direct damage to olfactory sensory neurons [36]. Furthermore, the distribution of these two receptors may form a plausible explanation for the spectrum of smell loss, with mild and typically short-lived smell loss from ACE-2 invasion versus more longstanding smell loss via NRPI mediated sensory neuronal damage, following COVID-19. Therapeutic strategies including olfactory rehabilitation and corticosteroids are already in use to aid recovery of smell function following COVID-19, with further therapeutic strategies in clinical trials [14, 15]. With emerging treatments for COVID-19 related anosmia and the high predicted prevalence of long covid with long-standing smell loss, identifying those at risk of long-COVID with smell loss will be key. We highlight female sex (and increasing age within female cohorts) as well as the presence of parosmia as key risk factors for a prolonged clinical course of COVID-19 related smell loss.

Finally, in this study, we addressed the limitation of using subjective assessment of smell by recruiting a subset of participants who underwent objective UPSIT testing. Our results show that objectively assessed smell function using UPSIT correlates well with perceived smell function in this population, which highlights the reliability of our subjective patient-reported data on smell function following COVID-19. This finding is in line with a study conducted in ambulatory COVID-19 patients using the 12-item Brief Smell Identification Test (BSIT) which also concluded that self-reported olfactory loss is a strong predictor of abnormal olfactory function [39]. Furthermore, this highlights that in this subsample, tested 21.6 ± 4.7 weeks since the onset of their symptoms, 20% of participants had ongoing loss of their sense of smell, who would meet diagnostic criteria for both long-COVID and chronic COVID-19.

Limitations
The main limitation of this study remains the lack of a general population control group without loss of smell/taste. Our selection criteria enabled us to study acute smell and taste loss as presentations of COVID-19 as well as their resolution in people both with and without SARS-CoV-2 antibodies. Coupled with the web-based delivery of the study, this leads to susceptibility to a degree of selection and age bias, which required proficiency with computers and smartphones and may have resulted in under-representation of older adults. Furthermore, the majority of our participants were female; this may reflect previous findings that females are more likely to engage in research and also have a higher frequency of loss of smell and/or taste with COVID-19 than males. Through recruiting a subset of participants for objective testing we addressed the previous limitation of lack of objective testing, and demonstrated a strong correlation between perceived and assessed smell function.

Conclusions
We followed up a community cohort of people who had reported acute loss of their sense of smell and/or taste and had undergone SARS-CoV-2 IgG/IgM antibody testing 4–6 weeks earlier in order to investigate the clinical course of smell and/or taste loss. We also aimed to identify factors that were associated with persistent smell loss at 4–6 weeks of follow-up. In line with existing literature, we can offer reassurance that smell and/or taste loss is a transient phenomenon in most SARS-CoV-2 cases. However, persistent smell and taste loss constitute a feature of long-COVID. The population of patients with longstanding smell and/or taste loss as a manifestation of long-COVID will continue to grow during and following the pandemic; given the impact of these symptoms on quality of life and safety, it will be imperative to devise support and treatment pathways. We identified the presence of parosmia and female sex as risk factors for persistent smell loss, as well as increasing age within the female sub-cohort. Similarly, female sex and increasing age as well as distorted taste perception were associated with persistent taste loss. Our findings highlight that female patients over the age of 40, who experience with a distorted perception of their sense of smell and/or taste are likely to benefit from therapeutic interventions to prevent persistent smell and/or taste loss and should be prioritized when targeted therapies for post-covid smell and taste loss become available.

Supplementary Information
The online version contains supplementary material available at https://doi.org/10.1186/s12879-021-05927-w.

Additional file 1. Study questionnaires.
Additional file 2. Demographics of participants with positive and negative SARS-CoV-2 antibodies from entire study cohort.

Abbreviations
SARS-CoV-2: Severe Acute Respiratory Syndrome Coronavirus 2; COVID-19: Coronavirus disease 2019; IgG: Immunoglobulin G; IgM: Immunoglobulin M; UPSIT: University of Pennsylvania Smell Identification Test; ACE-2: Angiotensin-converting enzyme-2 receptor; NRP1: Neuropilin-1 receptor; BSIT: Brief Smell Identification Test

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Authors’ contributions
JMJ: Conceptualization, Data curation, Participant recruitment, Formal analysis, Investigation, Validation, Writing; CF: Data curation, Formal analysis, Investigation, Writing; CM: Investigation, Methodology, Writing; MZ: Investigation, Methodology, Writing, NB: Data curation, Investigation, Writing; ML: Investigation, Writing; AC: Conceptualization, Formal analysis.
Investigation, Methodology, Project administration, Writing. RLB: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Resources, Writing. All authors read and approved the final manuscript.

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**Availability of data and materials**
The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

**Ethics approval and consent to participate**
The study received ethical approval from the National Health Service Queen’s Square Research Ethics Committee (IRAS Project ID 229909, ClinicalTrials.gov NCT02378715) and was conducted in line with the declaration of Helsinki and Good Clinical Practice. Participation in the study was voluntary and written informed consent was obtained electronically.

**Consent for publication**
Not applicable.

**Competing interests**
The authors declare no competing interests.

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