A comprehensive evaluation of the longitudinal association between alcohol consumption and a measure of inflammation: Multiverse and vibration of effects analyses

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ABSTRACT

Background: Moderate alcohol consumption appears to be associated with reduced inflammation. Determining whether this association is robust to common variations in research parameters has wide-reaching implications for our understanding of disease aetiology and public health policy. We aimed to conduct comprehensive multiverse and vibration of effects analyses evaluating the associations between alcohol consumption and a measure of inflammation.

Methods: A secondary analysis of the 1970 British Birth Cohort Study was performed, using data from 1970 through 2016. Measurements of alcohol consumption were taken in early/mid-adulthood (ages 34 and 42), and level of inflammation marker high-sensitivity C-reactive protein (hsCRP) at age 46. Multiverse analyses were applied to comparisons of low-to-moderate consumption and consumption above various international drinking guidelines with an 'abstinent' reference. Research parameters of interest related to: definitions of drinking and reference groups; alcohol consumption measurement year; outcome variable transformation; and breadth of covariate adjustment. After identifying various analytic options within these parameters and running the analysis over each unique option combination, specification curve plots, volcano plots, effect ranges, and variance decomposition metrics were used to assess consistency of results.

Results: A total of 3101 individuals were included in the final analyses, with primary analyses limited to those where occasional drinkers served as reference. All combinations of research specifications resulted in lower levels of inflammation amongst low-to-moderate drinkers compared to occasional drinkers (1^{st} percentile effect: -0.21; 99^{th} percentile effect: -0.04). Estimates comparing above-guidelines drinking with occasional drinkers were less definitive (1^{st} percentile effect: -0.26; 99^{th} percentile effect: 0.43).

Conclusions: The association between low-to-moderate drinking and lower hsCRP levels is largely robust to common variations in researcher-defined parameters, warranting further research to establish whether this relationship is causal. The association between above-guidelines drinking and hsCRP levels is less definitive.

KEYWORDS: Alcohol Drinking; Inflammation; C-Reactive Protein; hsCRP; Multiverse; Vibration of Effects

1 INTRODUCTION

Acute inflammation is a vital and adaptive response to pathogens and tissue injury. However, low-grade chronic inflammation, i.e., inflammation that fails to resolve or is continuously triggered, can negatively affect tissue and organs over time. This pathology ranges in severity, on the higher end associated with inflammatory diseases such as asthma, atherosclerosis, inflammatory bowel disease, and rheumatoid arthritis (Calder et al., 2013; Furman et al., 2019; Ridker, 2004). Inflammation is also increasingly implicated in a variety of other serious conditions including psychiatric disorders, cancer, type 2 diabetes, and cardiovascular disease (Ansar and Ghosh, 2013; Avan et al., 2018; Furman et al., 2019; Réus et al., 2015).

Of the many putative lifestyle influences on inflammation, there is strong evidence for the role of alcohol consumption. Alcohol use disorder is associated with increased levels of pro-inflammatory cytokines (Adams et al., 2020) as well as neuroinflammation specifically (de Timary et al., 2017; He and Crews, 2008). Harmful drinking is responsible for substantial disease burden globally (Bryazka et al., 2022), and there is evidence that inflammation may mediate the increased risk heavy drinking brings for a range of health outcomes (González-Reimers et al., 2014).

However, as for many conditions to which alcohol consumption is thought to contribute pathophysiologically (Visontay et al., 2022), some research suggests that compared to abstaining, lower levels of drinking may actually be beneficial when it comes to inflammation (Albert et al., 2003; Bell et al., 2017; Pai et al., 2006; Paulson et al., 2018; Volpato et al., 2004). This is typically indexed via inflammatory markers such as high-sensitivity C-reactive protein (hsCRP) – widely used by the clinical and research communities due to its stable expression in response to elevated pro-inflammatory cytokines (Coventry et al., 2009). Reduced inflammation has also been suggested as a partial mediator of the lower risk moderate drinking seemingly affords against conditions such as cardiovascular disease (Pai et al., 2006) and depression (Paulson et al., 2018).

However, there are major inconsistencies in the evidence base. For example, short-term alcohol administration tends to demonstrate no relationship with hsCRP (Brien et al., 2011). Several cohort studies have also failed to find a relationship between drinking and inflammation (Menezes et al., 2019; van't Klooster et al., 2020). A possible explanation for these conflicting findings is that data

processing and analysis choices, such as which covariates are controlled for, vary considerably between studies. A growing literature is beginning to uncover the impact these alternative specifications in research parameters, or 'researcher degrees of freedom', (Simmons et al., 2011) can have on effect estimates, challenging implied assumptions that these decisions are innocuous. To this end, three similar analytic frameworks have emerged to quantify sensitivity (or robustness) to alternative specifications: multiverse analysis (Steegen et al., 2016), specification curve analysis (Simonsohn et al., 2020), and vibration of effects (VoE) (Patel et al., 2015). These frameworks are an extension of standard sensitivity analyses, providing methods to comprehensively analyse and summarise the sensitivity of exposure–outcome associations to alternative specifications.

It is well documented that these alternative specifications can influence effect sizes, P-values, and even effect direction for a variety of epidemiological associations. This is becoming increasingly clear in alcohol–health associations specifically. Such parameters include how the abstaining reference group is defined (Stockwell et al., 2016), with increasing calls (but limited uptake) for occasional drinkers to be used as the reference given their greater prevalence and more normative risk factor profiles (Naimi et al., 2022). How alcohol intake is categorised, which covariates are adjusted for, and choice of modelling approach have also been identified as factors that may substantially impact findings in this space (Bell et al., 2017; Chu et al., 2020; Stockwell et al., 2016). It is thus concerning that there remains great variation in these parameters in the literature, and that their potential impact is rarely acknowledged in individual studies, where typically only one set of specifications are tested.

The present study aims to address this through systematically applying methods from enhanced sensitivity frameworks to test the alcohol–inflammation association. Using the 1970 British Birth Cohort Study (BCS70) – a source of rich, representative data – we assessed the relationship between alcohol consumption in early/mid-adulthood and hsCRP at age 46. The research parameters of interest explored here comprise: composition of reference group; classification of drinking groups according to various national guidelines ranging from conservative through liberal; measurement year(s) for alcohol consumption; transformation of the hsCRP outcome; and breadth of covariate adjustment. Each unique combination of specifications within these parameters constitutes a possible

'universe' within the analytic multiverse; here alcohol–hsCRP analyses are iterated over each universe and results interpreted on aggregate.

2. MATERIAL AND METHODS

Reporting for this study adheres to the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) guidelines (von Elm et al., 2007).

2.1 Data source and participants

BCS70 is a prospective cohort study targeting all individuals born in a single week in 1970 in Great Britain. It has had 11 sweeps to date, collecting diverse information on health, education, lifestyle behaviours, and socio-economic factors (Elliott and Shepherd, 2006). Individuals possessing valid alcohol data for at least one of ages 34 and 42, and an hsCRP measurement at age 46, were considered eligible for the present study.

2.2 Exposure

Self-reported alcohol consumption data at ages 34 (2004) and 42 (2012) were used to generate the exposure variables. In the present study, data on frequency and volume of alcohol consumption were converted to grams of alcohol per week, and then used to categorise individuals as either lifetime abstainers, former drinkers, occasional drinkers (less than weekly), low-to-moderate drinkers (at least once per week), or those consuming above various international drinking guidelines at each of the waves. Additional exposure variables were created re-categorising current drinkers according to their average weekly volume across the two ages, as well as their maximum weekly volume. Four separate versions were created for each of these four variables, where the threshold separating low-to-moderate from above-guidelines drinkers varied according to the national drinking guidelines of four chosen countries. See **Table 1** and **Supplementary Methods** for further detail.

2.3 Outcome

A non-fasting blood sample was taken at age 46 (2016), with hsCRP concentrations assessed via immunoturbidimetry (Hamer et al., 2020). Prior to analysis, 110 individuals with hsCRP concentrations > 10 mg L^{-1} were excluded, given these concentrations suggest acute inflammation or infection (Giollabhui et al., 2020). Chi-Square tests of independence revealed no relationship between drinking category at age 34 and likelihood of hsCRP being > 10 mg L^{-1}, although there was slight

evidence of a relationship between drinking category at age 42 and likelihood of hsCRP being > 10 mg L^{-1} (**Supplementary Table1**). The hsCRP distribution was positively skewed, so a natural log-transformed version was also created which led to an approximately normal distribution, and universes with both untransformed and log-transformed hsCRP were tested. Normality was assessed via visual inspection.

2.4 Covariates

Covariates were chosen *a prori* based on their relevance to the alcohol–inflammation relationship and were derived from data collected at either birth (for time-invariant variables) or age 30 (this was the nearest measurement occasion preceding age 34 alcohol consumption measurement, chosen to ensure covariates temporally preceded alcohol measurement). Covariates of interest were divided into five groups: demographic/socioeconomic, physical health, lifestyle behaviours, social, and mental health. See **Supplementary Methods** for further detail.

2.5 Parameters of interest and their specifications

As detailed in **Table 1**, the research parameters for which alternative specifications were explored were: 1) composition of 'abstinence' reference group (including using occasional drinkers instead of true abstainers); 2) the cut-point between low-to-moderate and above-guidelines drinking (based on four different national drinking guidelines ranging from conservative to liberal); 3) measurement year(s) of alcohol consumption predictor; 4) hsCRP outcome transformation; and 5) breadth of covariate adjustment. This produced a total of 1248 universes. Note that specification alternatives often resulted in sample size variation.

Table 1

Parameters and specifications tested.

Parameter	Specifications	*N* of specifications
Composition of 'abstinence' reference group	(i) Lifetime abstainers only (ii) Lifetime abstainers and former drinkers (iii) Occasional drinkers only	3
Cut-point for above-guidelines drinking	(i) Dutch guidelines (>70g/wk) (Health Council of the Netherlands, 2015) (ii) UK guidelines (>112g/wk) (Department of Health, 2016)	4

	(iii) US guidelines (F: >98g/wk; M: >196g/wk) (U.S. Department of Agriculture and U.S. Department of Health and Human Services, 2020) (iv) Former Spanish guidelines[a] (F: >170g/wk; M: >280g/wk) (Spanish Ministry of Health, 2020)	
Year of alcohol consumption measurement	(i) 2004 (ii) 2012 (iii) Maximum of 2004 and 2012 (iv) Average of 2004 and 2012	4
Transformation of hsCRP outcome	(i) Leave untransformed (ii) Natural log-transform	2
Breadth of covariate adjustment	(i) No covariates adjusted for (ii) Demographics (iii) Demographics + Physical health (iv) Demographics + Mental health (v) Demographics + Lifestyle (vi) Demographics + Social (vii) Demographics + Physical health + Mental health (viii) Demographics + Physical health + Lifestyle (ix) Demographics + Mental health + Lifestyle (x) Demographics + Mental health + Social (xi) Demographics + Physical health + Social (xii) Demographics + Lifestyle + Social (xiii) All covariates adjusted for	13
Total universes		1248

The total number of universes is equal to the product of the five parameters' specification *N*s.
[a] These guidelines are no longer in effect as of 2020, but are indicative of liberal thresholds for 'moderate consumption' adopted by various policymakers and researchers.

2.6 Statistical analysis

A linear regression of continuous hsCRP levels on categorical alcohol consumption was performed for all 1248 universes, producing two comparisons: 'abstinence' vs. low-to-moderate alcohol consumption, and 'abstinence' vs. above-guidelines alcohol consumption. Effect sizes were standardised.

Various approaches from the sensitivity frameworks introduced earlier were employed to interpret results. Specification curve plots allowed for visual inspection of variation in effect sizes and P-values across universes. In these plots, universes are arranged in order of effect size, with an accompanying panel depicting the corresponding specifications. Results were also graphically depicted using volcano plots, which promote evaluation of (in)consistency of effect direction (Klau et al., 2021). Metrics developed in the VoE framework were also calculated: the Range of Betas (RBs)

and Range of P-values (RPs). These are simple figures calculated as the range of standardised betas and P-values between the 1st and 99th percentiles, where larger ranges indicate greater variability across universes (Patel et al., 2015).

Additionally, variance in effect estimates were decomposed by parameter, and each specification plotted against effect estimates in box and whisker plots. All analyses were conducted using R version 4.1.3 with packages *multiverse* (Sarma, 2021) and *specr* (Masur and Scharkow, 2020).

3 RESULTS

3.1 Descriptive characteristics

In total, 11 123 individuals provided valid age 34 and/or age 42 alcohol data, with 3211 of these also having age 46 hsCRP data. The small size of the latter figure owed to attrition and low health test completion rates, although drinking status and covariates were largely unassociated with providing hsCRP data (**Supplementary Table2**). After removing those with elevated hsCRP, the final sample was 3101.

Compared to other categories, low-to-moderate drinkers were more likely to rate their health as excellent, and had lower mean Malaise Inventory and hsCRP values (**Table 2** and **Supplementary Table3**). There was considerable movement amongst drinking categories between ages 34 and 42, particularly a decrease in individuals drinking above-guidelines and an increase in those drinking occasionally.

Table 2

Cohort characteristics for selected covariates and hsCRP by age 34 alcohol consumption categories based on UK guidelines

	Lifetime abstainer (N=52)	Former drinker (N=108)	Occasional drinker (N=621)	Low-to-moderate drinker (N=1160)	Above-guidelines drinker (N=684)	Total (N=2625)
UK standard drinks per week						
Mean (SD)	NA (NA)	NA (NA)	NA (NA)	6.99 (3.68)	29.8 (18.6)	15.5 (16.1)
Median [Min, Max]	NA [NA, NA]	NA [NA, NA]	NA [NA, NA]	6.00 [1.00, 14.0]	24.0 [15.0, 280]	10.0 [1.00, 280]
Missing	52 (100%)	108 (100%)	621 (100%)	0 (0%)	0 (0%)	781 (29.8%)
Sex						
Female	28 (53.8%)	71 (65.7%)	406 (65.4%)	686 (59.1%)	154 (22.5%)	1345 (51.2%)
Male	24 (46.2%)	37 (34.3%)	215 (34.6%)	474 (40.9%)	530 (77.5%)	1280 (48.8%)
Mother's age at birth						
Mean (SD)	28.1 (5.78)	25.1 (4.72)	25.9 (5.23)	26.4 (5.14)	26.1 (5.22)	26.2 (5.19)
Median [Min, Max]	28.0 [17.0, 41.0]	25.0 [16.0, 38.0]	25.0 [15.0, 44.0]	26.0 [15.0, 43.0]	25.0 [16.0, 49.0]	25.0 [15.0, 49.0]
Missing	12 (23.1%)	11 (10.2%)	35 (5.6%)	77 (6.6%)	48 (7.0%)	183 (7.0%)
Self-rated health (Age 30)						
Excellent	16 (30.8%)	25 (23.1%)	185 (29.8%)	411 (35.4%)	200 (29.2%)	837 (31.9%)
Good	25 (48.1%)	60 (55.6%)	322 (51.9%)	590 (50.9%)	355 (51.9%)	1352 (51.5%)
Fair	6 (11.5%)	14 (13.0%)	79 (12.7%)	96 (8.3%)	87 (12.7%)	282 (10.7%)
Poor	2 (3.8%)	5 (4.6%)	13 (2.1%)	6 (0.5%)	5 (0.7%)	31 (1.2%)

	Lifetime abstainer (N=52)	Former drinker (N=108)	Occasional drinker (N=621)	Low-to-moderate drinker (N=1160)	Above-guidelines drinker (N=684)	Total (N=2625)
Missing	3 (5.8%)	4 (3.7%)	22 (3.5%)	57 (4.9%)	37 (5.4%)	123 (4.7%)
BMI (Age 30)						
Mean (SD)	24.2 (4.27)	24.8 (5.48)	25.2 (4.81)	24.2 (4.09)	25.0 (3.44)	24.7 (4.21)
Median [Min, Max]	24.0 [18.1, 40.2]	23.6 [16.9, 45.2]	24.1 [16.4, 57.2]	23.4 [11.1, 61.2]	24.9 [9.98, 44.9]	24.0 [9.98, 61.2]
Missing	5 (9.6%)	9 (8.3%)	43 (6.9%)	83 (7.2%)	47 (6.9%)	187 (7.1%)
Smoking status (Age 30)						
Never smoker	37 (71.2%)	47 (43.5%)	304 (49.0%)	548 (47.2%)	241 (35.2%)	1177 (44.8%)
Former smoker	3 (5.8%)	18 (16.7%)	89 (14.3%)	253 (21.8%)	144 (21.1%)	507 (19.3%)
Occasional smoker	0 (0%)	9 (8.3%)	34 (5.5%)	94 (8.1%)	69 (10.1%)	206 (7.8%)
Daily smoker	9 (17.3%)	30 (27.8%)	172 (27.7%)	208 (17.9%)	193 (28.2%)	612 (23.3%)
Missing	3 (5.8%)	4 (3.7%)	22 (3.5%)	57 (4.9%)	37 (5.4%)	123 (4.7%)
Weekly exercise (Age 30)						
No	14 (26.9%)	29 (26.9%)	213 (34.3%)	295 (25.4%)	172 (25.1%)	723 (27.5%)
Yes	35 (67.3%)	74 (68.5%)	386 (62.2%)	808 (69.7%)	475 (69.4%)	1778 (67.7%)
Missing	3 (5.8%)	5 (4.6%)	22 (3.5%)	57 (4.9%)	37 (5.4%)	124 (4.7%)
Malaise scale total (Age 30)						
Mean (SD)	3.65 (3.73)	4.23 (3.69)	3.61 (3.48)	2.93 (3.06)	3.30 (3.05)	3.26 (3.22)
Median [Min, Max]	3.00 [0, 15.0]	3.00 [0, 16.0]	3.00 [0, 18.0]	2.00 [0, 21.0]	2.00 [0, 22.0]	2.00 [0, 22.0]

	Lifetime abstainer (N=52)	Former drinker (N=108)	Occasional drinker (N=621)	Low-to-moderate drinker (N=1160)	Above-guidelines drinker (N=684)	Total (N=2625)
Missing	3 (5.8%)	5 (4.6%)	25 (4.0%)	67 (5.8%)	41 (6.0%)	141 (5.4%)
Ever member of communal organisation (Age 30)						
No	34 (65.4%)	86 (79.6%)	461 (74.2%)	838 (72.2%)	553 (80.8%)	1972 (75.1%)
Yes	15 (28.8%)	17 (15.7%)	138 (22.2%)	265 (22.8%)	94 (13.7%)	529 (20.2%)
Missing	3 (5.8%)	5 (4.6%)	22 (3.5%)	57 (4.9%)	37 (5.4%)	124 (4.7%)
hsCRP (log-transformed) (Age 46)						
Mean (SD)	-0.122 (3.04)	-0.0338 (2.27)	0.0187 (2.12)	-0.312 (2.80)	-0.0637 (1.67)	-0.154 (2.38)
Median [Min, Max]	0.0953 [-20.7, 2.16]	0.182 [-20.7, 2.30]	0.0953 [-20.7, 2.24]	-0.105 [-20.7, 2.29]	-0.105 [-20.7, 2.30]	0 [-20.7, 2.30]

Abbreviations: SD, standard deviation; BMI, body mass index; hsCRP, high-sensitivity C-reactive protein.
See **Supplementary Table3** for characteristics by age 42 drinking categories.

3.2 Summary of effects and their heterogeneity

Despite a considerable range of effects (RB=0.46; RP=4.03), results favoured lower levels of hsCRP in low-to-moderate drinkers compared to the 'abstainer' reference, with effects at both the 1st (-0.50) and 99th (-0.05) percentiles in this direction. None of the 1248 estimates were consistent with increased hsCRP levels (**Supplementary Figures 1 and 2**). Estimates comparing above-guidelines drinking with 'abstainers' were less definitive (RB=0.86; RP=2.29; **Supplementary Figure 3 and 4**), with 136/1248 estimates consistent with increased hsCRP levels, and the effects at the 1st (-0.47) and 99th (0.39) percentiles being in opposite directions. **Supplementary Figures 1 and 3** reveal considerable variation in effects according to reference group composition (lifetime abstainers and former drinkers; lifetime abstainers only; occasional drinkers), making it difficult to interpret other parameters.

Occasional drinkers proved the only appropriate reference given the very small sample size resulting from the other two reference specifications. Analyses with this specification were thereafter considered the primary analyses, to mitigate confounding by sample size and aid interpretability (**Figures 1 and 2**; **Supplementary Figures 5 and 6**).

The range of effects in these analyses was narrower, with RBs of 0.16 (RP= 4.65) and 0.69 (RP= 2.87) for the low-to-moderate (0/416 estimates consistent with increased hsCRP; 1st percentile effect: -0.21; 99th percentile effect: -0.04) and above-guidelines (85/416 estimates consistent with increased hsCRP; 1st percentile effect: -0.26; 99th percentile effect: 0.43) comparisons respectively.

[FIGURE 1 ABOUT HERE]

[FIGURE 2 ABOUT HERE]

3.3 Variance decomposition and impact of individual specifications

Using a multi-level model with random effects for each parameter of interest, the highest intraclass correlation coefficient (ICC) for the low-to-moderate drinkers vs occasional drinkers comparison belonged to breadth of covariate control (0.20), followed by which national guidelines were used to define drinking categories (0.13). The highest ICC for the above-guidelines vs

occasional drinkers comparison belonged to measurement year that drinking category was based on (0.23), followed by which national guidelines were used to define drinking categories (0.14).

Box and whisker plots (**Figure 3**) offer insights into the impact of particular specifications. Mean protective effects were greater when the ceiling for low-to-moderate drinking was lower. Conversely, the Spanish-based categorisation of above-guidelines drinking – i.e., when this group was restricted to those drinking most heavily – resulted in the smallest mean protective effect. **Figure 3** also shows that protective effects of above-guidelines consumption appear when considering drinking at age 34 only (12 year before hsCRP measurement) but not drinking at age 42 only (four years before hsCRP measurement).

[FIGURE 3 ABOUT HERE]

3.3 Post-hoc sensitivity analyses

The multiverse analyses were re-run on a reduced sample, restricted to individuals with no missing covariate data (n=1358). Results were consistent with the main analyses (see **Supplementary Methods** and **Supplementary Figures 7-9**).

4 DISCUSSION

As inconsistencies in the existing evidence base would suggest, this analysis revealed substantial heterogeneity, or effect vibration, in estimates of the alcohol–hsCRP relationship when research parameter specifications are varied. Composition of the 'abstinence' reference group had the clearest impact on effect estimates. When analyses were restricted to universes with occasional drinkers as reference, additional impacts of the breadth of covariate adjustment (for the low-to-moderate drinking comparison), and measurement year and national guidelines used to classify drinking groups (for the above-guidelines drinking comparison), were apparent.

However, despite heterogeneity in effect and P-value size, the direction of results from comparisons of low-to-moderate alcohol consumption with 'abstinence' was robust to parameter specifications. Results were consistent with lower inflammation in the low-to-moderate alcohol

consumption group at follow-up. While this does not substantiate a causal role of moderate drinking in reducing inflammation, it fulfils a prerequisite. That is, before determining whether an observed association is causal, that association must be confirmed as a robust one that holds across methodological variations – what has been labelled the 'consistency' criteria for causation. (Hill, 1965)

Plausible biological mechanisms that may account for this association have been suggested, largely derived from short-term administration studies in animal and human models, and *in vitro* work. These centre on low-dose ethanol's ability to inhibit pro-inflammatory cytokines and chemokines (Muralidharan et al., 2014; Szabo, 2007). Additionally, given the established impact of chronic stress on inflammation (Rohleder, 2019), moderate drinking may exert a protective effect mediated by stress reduction.

Interestingly, previous research has shown that excessive alcohol use begins to exert opposite effects on these mechanisms, promoting pro-inflammatory responses (Szabo, 2007). As such, it is ostensibly surprising that the above-guidelines comparisons were less definitive. In fact, those effects with smaller P-values belonged to the analyses in which above-guidelines drinking were associated with lower levels of inflammation compared to the reference. Importantly though, the above-guidelines category did not reflect disordered/excessive drinking specifically, which is difficult to capture in general population samples. Given most individuals in this category were not drinking substantially over guidelines (**Supplementary Table4**), they may still have been drinking at levels below those at which ethanol promotes inflammation. Indeed, specifying above-guidelines drinking cut-points based on Spanish guidelines – i.e., restricting this group to those drinking most heavily – resulted in a bimodal split in effect direction (**Figure 2**), and the only interquartile effect range *not* situated entirely in the direction of reduced inflammation (**Figure 3**).

4.1 Strengths and limitations

To the authors' knowledge, this is the first study to examine the longitudinal association between alcohol consumption and a measure of inflammation via an enhanced sensitivity analysis framework. Previously, sensitivity considerations had only been explored in limited, disparate ways. The focus of the present study was on key parameters that researchers must consider carefully when

engaging in study design and analysis – those which existing evidence indicates stand to impact results and for which diverging specifications are typical in the literature. The present findings stand to inform both the evidence base on the alcohol–inflammation relationship as well as future research methodology.

A further strength of this study was the large sample size and the rich covariate selection available, including BMI and pre-existing inflammatory conditions. Age, a key determinant in inflammation, was held constant by design of the cohort. Sensitivity analyses of individuals with full covariate data were consistent with the main analyses, increasing confidence that findings were not confounded by sample variation between universes.

However, a limitation of this work was that the clear effects of reference group composition (**Supplementary Figures 1 and 3**) could not be confirmed to be a result of the characteristics of the groups themselves, as opposed to the small number of lifetime abstainers and former drinkers. Additionally, because BCS70 tested only a single inflammatory marker, the present analyses were restricted to hsCRP alone as a proxy for inflammation. This limits our ability to generalise results to inflammation more broadly; it is possible that other measures may have shown discrepant results (e.g., differences may be evident between alcohol–CRP and alcohol– interleukin-6 (IL-6) relationships (Silverwood et al., 2014)).

Measurement error may also have impacted the findings; while we removed cases with indications of acute inflammation, promoting a focus on chronic low-grade inflammation, hsCRP was only captured at a single time point. Additionally, the effect of certain covariates on hsCRP levels may have been larger had there been a shorter interval between their relative assessments. Also, most data were collected via self-report, which is problematic for the kinds of health covariates employed here (although it is the chief measurement modality in the literature). Further measurement bias was possible given the considerable movement between drinking categories from age 34 to age 42 – possibly explained by UK policy changes beginning in 2003 (Nicholls, 2012) as well as natural 'maturing out' of drinking – suggesting hidden 'drinker drift' in the interim between ages 42 and hsCRP measurement.

Finally, exploring additional parameters of interest, e.g., predominant beverage consumed, proved difficult within the multiverse framework. Although given suggested protective mechanisms operate via ethanol itself (Krenz and Korthuis, 2012) – common to all alcoholic drinks – this should not have considerably impacted results.

4.2 Implications

Given the present evidence of a robust association between low-to-moderate drinking and reduced hsCRP, future work should investigate whether this relationship is causal. One such avenue is non-linear Mendelian randomisation; to date one study has found a small protective effect for low-level drinking against CRP, but a positive linear relationship between alcohol and IL-6(Silverwood et al., 2014). Causal mediation analyses should also be undertaken to test the hypothesis that inflammation may mediate the relationship between moderate drinking and reduced cardiovascular disease risk. Were moderate alcohol consumption found to have a causal role in lowering inflammation, this would be important information for clinicians and policymakers, including for the generation of low-risk drinking guidelines. However, these findings would have to be balanced against the known harms to other organ systems/disease processes that accrue at even low-level drinking, such as increased cancer risk (Rehm et al., 2019). While results from universes with and without adjustment for sex did not vary substantially, sex-specific differences in the alcohol–inflammation relationship should also be explored in future work.

The present findings lend support to using occasional/very low volume drinkers as the reference group in future work. While this requires the assumption that such infrequent alcohol consumption would be unlikely to have a biological effect, the present study demonstrates clear benefits in the way of increased statistical power and more normative covariate profiles compared with lifetime abstainers and former drinkers. Results derived using occasional drinker reference groups are also less likely to be contaminated by ‘sick quitters’ (those for whom illness precipitates abstinence), former drinkers misidentified as lifetime abstainers, or hidden illness belonging to those excluded from the reference group (Naimi et al., 2022).

5 CONCLUSIONS

The present study supports a small but robust association between low-to-moderate alcohol consumption and lower levels of the inflammatory marker hsCRP, while the association between drinking at above-guidelines levels and hsCRP demonstrated greater variability. Further research using methodologies that promote causal inference is required. Common variations in research parameters in this field – such as composition of reference group, how drinking groups are defined, and which covariates are controlled for – are *not* innocuous, and must be considered carefully.

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FIGURE CAPTIONS

Figure 1. Specification curve plot of estimates comparing low-to-moderate drinkers with a reference group composed of occasional drinkers.

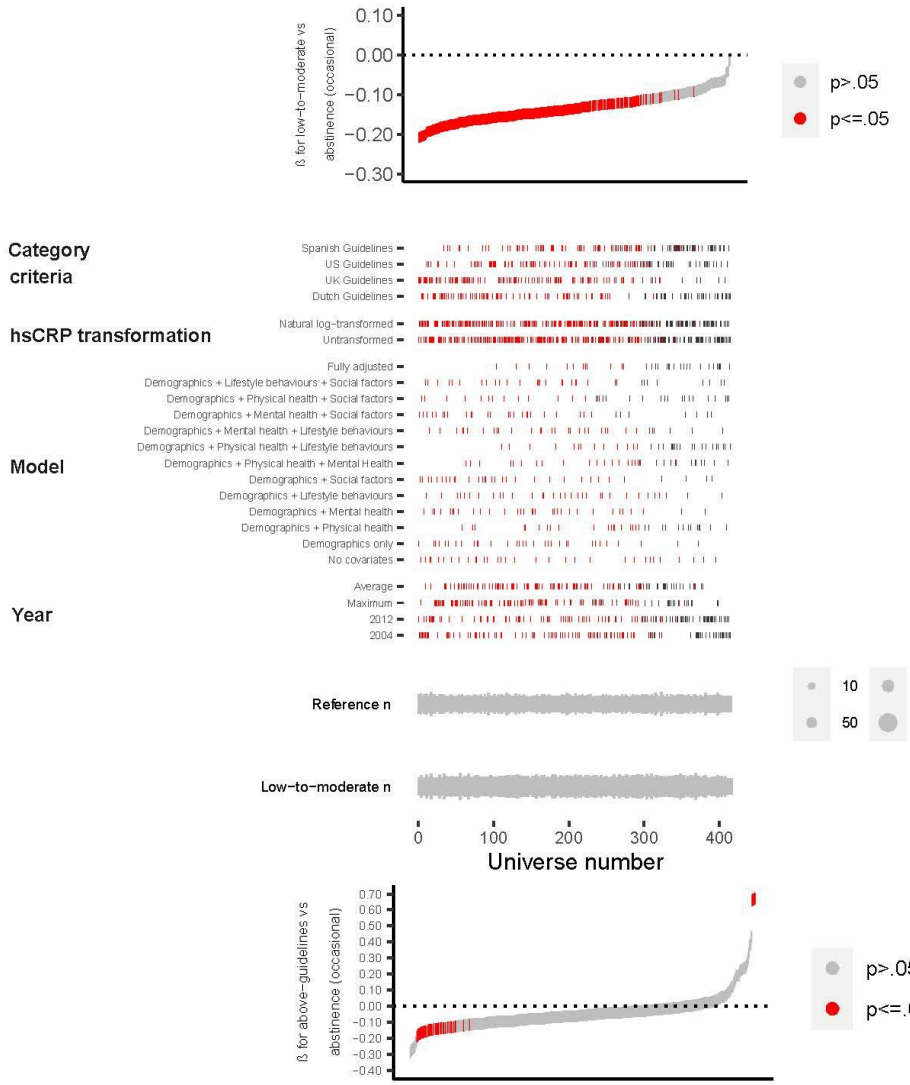
The top section displays the results associated with each universe, ordered by effect size. Negative values indicate drinking is associated with lower hsCRP. The middle section shows which specifications correspond to each result in the top panel. The bottom section displays the number of individuals in the reference and comparator groups in each universe.

Figure 2. Specification curve plot of estimates comparing above-guidelines drinkers with a reference group composed of occasional drinkers.

The top section displays the results associated with each universe, ordered by effect size. Negative values indicate drinking is associated with lower hsCRP. The middle section shows which specifications correspond to each result in the top panel. The bottom section displays the number of individuals in the reference and comparator groups in each universe.

Figure 3. Box and whisker plot depicting impact of specifications on comparisons between low-to-moderate/above-guidelines alcohol consumption and occasional drinking.

Red dots represent outliers.


β for low-to-moderate vs abstinence (occasional)
0.10
0.00
−0.10
−0.20
−0.30
p>.05
p<=.05
Category criteria
Spanish Guidelines
US Guidelines
UK Guidelines
Dutch Guidelines
hsCRP transformation
Natural log-transformed
Untransformed
Model
Fully adjusted
Demographics + Lifestyle behaviours + Social factors
Demographics + Physical health + Social factors
Demographics + Mental health + Social factors
Demographics + Mental health + Lifestyle behaviours
Demographics + Physical health + Lifestyle behaviours
Demographics + Physical health + Mental Health
Demographics + Social factors
Demographics + Lifestyle behaviours
Demographics + Mental health
Demographics + Physical health
Demographics only
No covariates
Year
Average
Maximum
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Low-to-moderate n
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100
500
1000
2000
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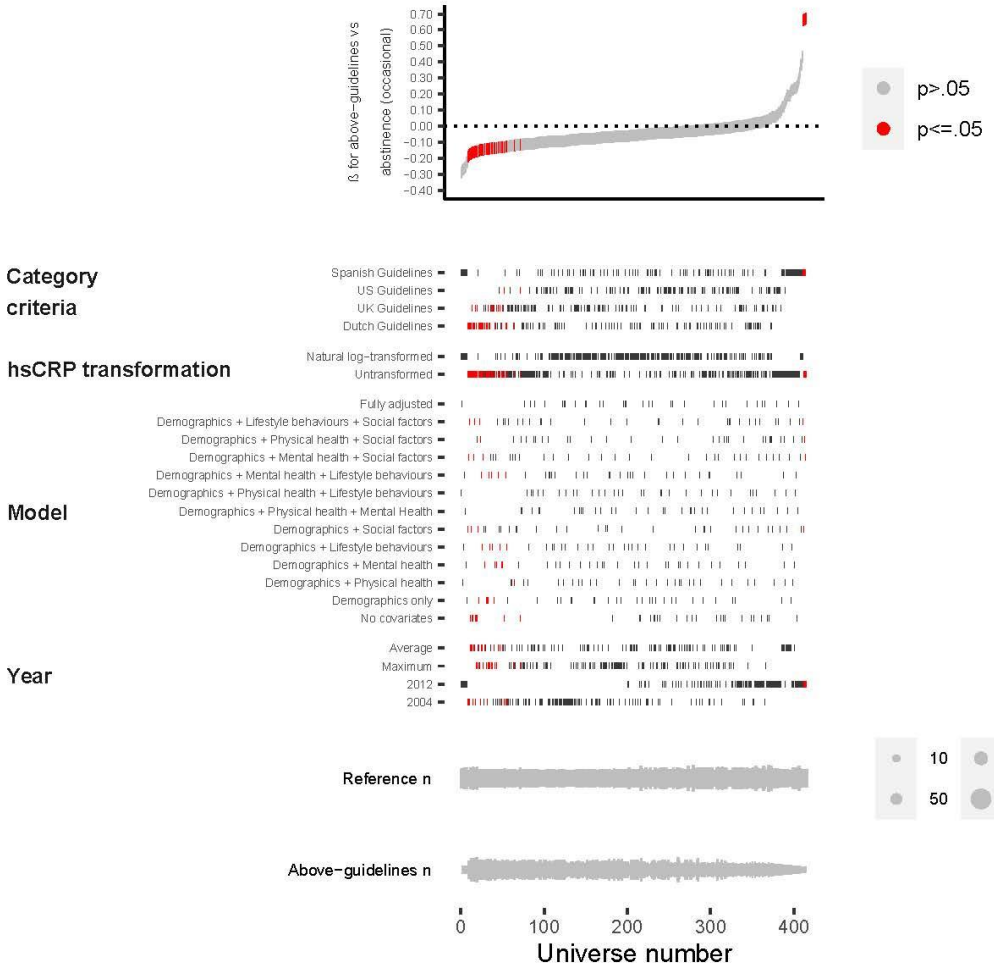
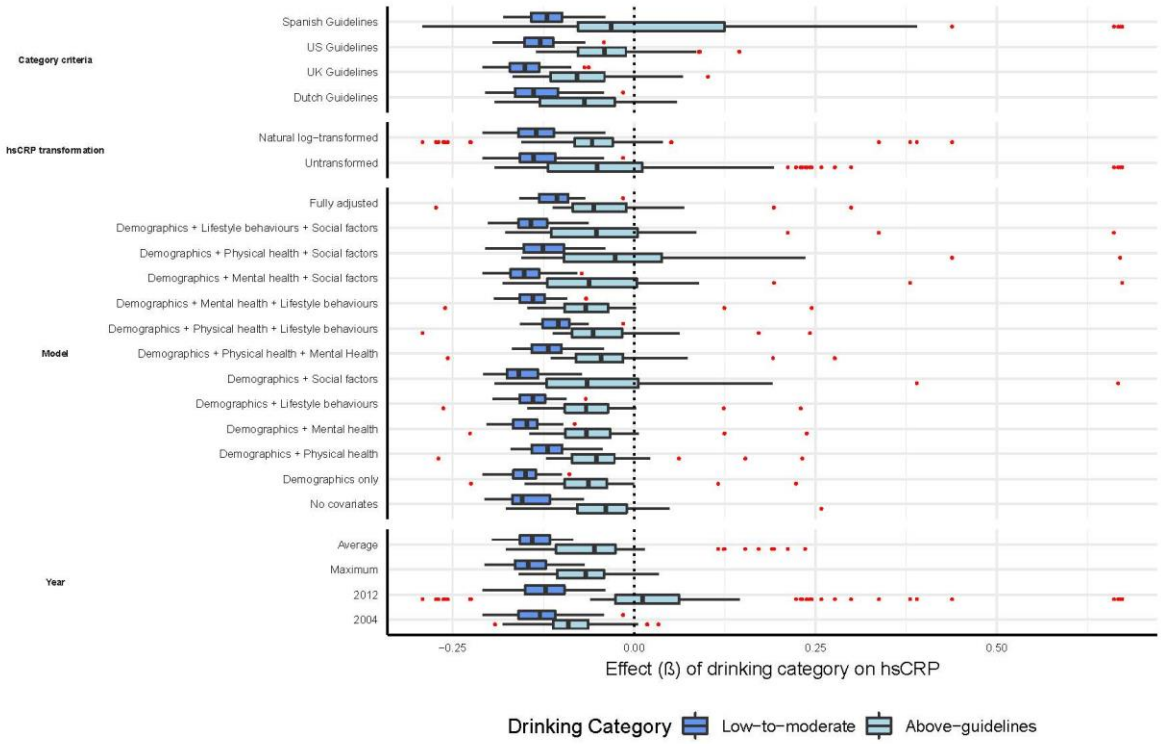
100
200
300
400
Universe number


β for above-guidelines vs abstinence (occasional)
0.70
0.60
0.50
0.40
0.30
0.20
0.10
0.00
−0.10
−0.20
−0.30
−0.40
p>.05
p<=.05
Category criteria
Spanish Guidelines
US Guidelines
UK Guidelines
Dutch Guidelines
hsCRP transformation
Natural log-transformed
Untransformed
Model
Fully adjusted
Demographics + Lifestyle behaviours + Social factors
Demographics + Physical health + Social factors
Demographics + Mental health + Social factors
Demographics + Mental health + Lifestyle behaviours
Demographics + Physical health + Lifestyle behaviours
Demographics + Physical health + Mental Health
Demographics + Social factors
Demographics + Lifestyle behaviours
Demographics + Mental health
Demographics + Physical health
Demographics only
No covariates
Year
Average
Maximum
2012
2004
Reference n
Above-guidelines n
10
50
100
500
1000
2000
0
100
200
300
400
Universe number


Category criteria
Spanish Guidelines
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hsCRP transformation
Natural log−transformed
Untransformed
Model
Fully adjusted
Demographics + Lifestyle behaviours + Social factors
Demographics + Physical health + Social factors
Demographics + Mental health + Social factors
Demographics + Mental health + Lifestyle behaviours
Demographics + Physical health + Lifestyle behaviours
Demographics + Physical health + Mental Health
Demographics + Social factors
Demographics + Lifestyle behaviours
Demographics + Mental health
Demographics + Physical health
Demographics only
No covariates
Year
Average
Maximum
2012
2004
−0.25
0.00
0.25
0.50
Effect (ß) of drinking category on hsCRP
Drinking Category
Low−to−moderate
Above−guidelines