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Long chain ω-3 levels are associated with increased alcohol sensitivity in a population-based sample of adolescents

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Abstract

Background

Levels of the ω-3 long-chain polyunsaturated fatty acids (ω–3 LC-PUFAs), including eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), have been associated with alcohol sensitivity in vertebrate and invertebrate model systems, but prior studies have not examined this association in human samples despite evidence of associations between ω–3 LC-PUFA levels and alcohol-related phenotypes. Both alcohol sensitivity and ω–3 LC-PUFA levels are impacted by genetic factors, and these influences may contribute to observed associations between phenotypes. Given the potential for using EPA and DHA supplementation in adjuvant care for alcohol misuse and other outcomes, it is important to clarify how ω–3 LC-PUFA levels relate to alcohol sensitivity.

Methods

Analyses were conducted using data from the Avon Longitudinal Study of Parents and Children (ALSPAC). Plasma ω–3 LC-PUFA levels were measured at ages 15.5 and 17.5. Participants reported on their initial alcohol sensitivity using the early drinking Self-Rating of Effects of Alcohol (SRE-5) scale, for which more drinks needed for effects indicates lower levels of response per drink, at ages 15.5, 16.5, and 17.5. Polygenic liability for alcohol consumption, alcohol problems, EPA levels, and DHA levels were derived using summary statistics from large, publicly available datasets. Linear regressions were used to examine the cross-sectional and longitudinal associations between ω–3 LC-PUFA levels and SRE scores.

Results

Age 15.5 ω–3 LC-PUFA levels were negatively associated with contemporaneous SRE scores and with age 17.5 SRE scores. One modest association (p=0.02) between polygenic liability and SRE scores

was observed, between alcohol problems-based PRS and age 16.5 SRE scores. Tests of moderation by genetic liability were not warranted.

Conclusions

Plasma ω–3 LC-PUFA levels may be related to initial sensitivity to alcohol during adolescence. These data indicate that diet-related factors have the potential to impact humans' earliest responses to alcohol exposure.

Key words: ALSPAC, ω–3 long-chain polyunsaturated fatty acids, alcohol sensitivity, PUFA, HUFA, eicosapentaenoic acid, docosahexaenoic acid

Introduction

Alcohol problems are common in the US and other Western societies: A 2015 study of a population-based adult cohort reported a lifetime prevalence of 29.1% for DSM-5 alcohol use disorder (AUD) (Grant et al., 2015) and the World Health Organization estimated that 16.0% of drinkers aged 15 and older engage in heavy episodic drinking (World Health Organization, 2014). Due to the impact of excessive alcohol use on health and productivity, the economic consequences are substantial, estimated at $249 billion in 2010 in the US alone (Sacks et al., 2015). Clarifying the biological and environmental factors that contribute to the risk of developing alcohol problems is, therefore, a public health priority.

Long-chain ω–3 polyunsaturated fatty acid (ω–3 LC-PUFA; also known as highly unsaturated fatty acids [HUFA]) levels are a primarily environmental factor that has been associated with acute ethanol response behaviors in both invertebrate and vertebrate models. In *C. elegans,* genetic contributors to a low level of response (low LR) to alcohol have been identified (Davies et al., 2003), and the development of acute functional tolerance (AFT) to ethanol requires the long chain ω–3 eicosapentaenoic acid (EPA) (Raabe et al., 2014). Supplementation of additional EPA can enhance AFT, indicating that EPA levels can influence the acute response to ethanol in *C. elegans* (Raabe et al., 2014). In mice, dietary levels of long chain ω–3s interact with the genetic background to alter several acute ethanol responses including low dose locomotor activation and high dose sedation. Intriguingly, in C57BL/6J mice but not DBA/2J mice, dietary EPA and DHA increased voluntary ethanol consumption (Wolstenholme et al., 2018)

Acute ethanol response behaviors, including AFT, in model organisms are a model of initial alcohol sensitivity in humans. The initial acute physiological sensitivity to alcohol is a partially

heritable phenotype (Edwards et al., 2018, Heath et al., 1999, Schuckit, 2018) which has been associated with later alcohol consumption and problems (Schuckit, 1994, Schuckit et al., 2007). Lower initial sensitivity to alcohol is a risk factor for higher alcohol consumption and subsequent problems. Improved understanding of factors that impact one's alcohol sensitivity may therefore be useful in understanding trajectories from early to problematic alcohol use and has been used as a focus for prevention programs (Schuckit et al., 2016).

The effect of ω–3 LC-PUFA levels on the physiological response to ethanol may be of particular relevance to human alcohol use: Human EPA and DHA levels are primarily determined by diet, making them an easily modifiable target for alcohol studies. Indeed, EPA and DHA supplementation, usually from fish oil, is common, and can have significant impacts on the levels of ω–3 LC-PUFAs in plasma (Superko et al., 2013). However, in humans, little is known about how the levels of ω–3 LC-PUFAs may be related to alcohol sensitivity.

Here, we sought evidence for a relationship between measured plasma ω–3 LC-PUFA levels and alcohol sensitivity in the Avon Longitudinal Study of Parents and Children (ALSPAC). We capitalized on the availability of repeated measures of plasma ω–3 LC-PUFA levels and self-reported initial sensitivity to alcohol (SRE-5) across adolescence to test whether these measures were related, and if so, if the relationship was contemporaneous and/or whether ω–3 LC-PUFA levels are associated with later alcohol sensitivity. Prior evidence indicates that both initial alcohol sensitivity and ω–3 LC-PUFA levels are genetically influenced (Edwards et al., 2018, Lemaitre et al., 2011, Steer et al., 2012). The aforementioned differences in the phenotypic association between ω–3 LC-PUFA levels and alcohol consumption as a function of genetic background in mice (Wolstenholme et al., 2018) raises the question of whether genetic factors may have a similar impact in humans. We

therefore further assessed if polygenic liability for alcohol-related phenotypes and/or ω–3 LC-PUFA blood levels contributes to any association between ω–3 levels and alcohol sensitivity. Incorporation of aggregate genetic factors may clarify models of biological mechanism(s) contributing to the relationship between ω–3 LC-PUFA levels and alcohol outcomes. Furthermore, should genetic factors prove influential in this association, they could potentially inform the suitability of using EPA and DHA supplements in treatment settings.

Materials and Methods

Sample

There were 14,541 initial pregnancies for which the mothers enrolled in the Avon Longitudinal Study of Parents and Children (ALSPAC) study and had either returned at least 1 questionnaire or attended a "Children in Focus" clinic by July 19, 1999. Of these initial pregnancies, there was a total of 14,062 live births and 13,988 children who were alive at 1 year of age. Subsequent phases of enrollment increased the sample size over time (Fraser et al., 2013, Boyd et al., 2013). The phases of enrollment are described in more detail elsewhere (Fraser et al., 2013, Boyd et al., 2013). Only offspring genotypes were used in the current analyses. Participants are encouraged to contribute to assessments whenever possible even if not at every wave, and are permitted to skip questions within an assessment; accordingly, there is often variation across and within waves with respect to data availability for a given participant. The study website contains details of all the data that is available through a fully searchable data dictionary (http://www.bristol.ac.uk/alspac/researchers/our-data/). Beginning with the age 22 assessment, online questionnaires were administered using REDCap (Harris et al., 2009). Ethical approval for the study

was obtained from the ALSPAC Ethics and Law Committee and the Local Research Ethics Committees. Informed consent for the use of data collected via questionnaires and clinics was obtained from participants following the recommendations of the ALSPAC Ethics and Law Committee at the time.

Alcohol sensitivity

Sensitivity to alcohol was assessed using the Self-Rating of the Effects of Alcohol (SRE) scale (Schuckit et al., 1997). The SRE consists of 4 items; for the current study, each item referred to the *first five or so times*, the SRE-5, a participant used alcohol (referred to hereafter as SRE). Participants were asked to report the number of standard drinks they usually needed to consume to experience any effect of the alcohol, slur their speech, feel unsteady on their feet, or intentionally fall asleep. Consistent with prior reports (Edwards et al., 2018) responses were winsorized to limit extreme values and reduce the effect of possibly spurious outliers. SRE scores were calculated by summing the drinks needed for effects across items and dividing by the number of the up to four effects experienced, as recommended by Schuckit and colleagues (Schuckit et al., 1997). Thus, *higher SRE scores* correspond to *lower initial alcohol sensitivity*. The current study included SRE reports from approximate ages 15.5 (n=3285), 16.5 (n=1398), and 17.5 (n=942), which correspond to a time frame during which initiation of alcohol use is common and thus increases the likelihood that participants are reporting on their first experiences with alcohol.

Because we were interested in relationships between *initial* alcohol sensitivity and ω–3 levels, data were coded such that only a participant's first report of sensitivity was used. That is, if a participant responded to the SRE questionnaire items at ages 15.5 and 16.5, only the age 15.5 response was included in regressions; this decision was made to increase the likelihood that scores more closely

reflected the participant's first exposure to five or so drinks, as we were concerned that recall bias and/or more recent alcohol use experiences may impact responses during later assessments. Due to attrition and the fact that most participants had initiated alcohol use prior to age 16.5, a consequence of this decision was a smaller sample size in analyses for which the outcome was SRE score at age 16.5 or 17.5.

ω–3 LC-PUFA blood levels

ALSPAC participants periodically participate in clinics wherein physiological measures are taken in addition to typical questionnaire assessments. Fasting (minimum of 6 hours) plasma lipids were assessed at participant ages 15.5 (n=3361) and 17.5 (n=3166) (an assessment at age 7.5 was excluded as it was temporally distant from SRE reports). Lipids were measured using a high-throughput nuclear magnetic resonance metabolomics platform (Soininen et al., 2015, Soininen et al., 2009). We restricted our analyses to total ω–3 LC-PUFAs; data are in mmol/L.

Polygenic liability

To assess whether genetic factors relevant to alcohol outcomes and/or ω–3 LC-PUFA are related to any observed association between ω–3 LC-PUFA levels and initial alcohol sensitivity, we constructed polygenic risk scores for individuals within ALSPAC. PRS are derived by multiplying beta estimates (or odds ratios) for an effect allele at a particular locus – estimated in an independent sample – by the number of effect alleles an individual carries at that locus. This is repeated at the genome-wide level (after accounting for linkage disequilibrium). Ultimately, an individual's score reflects their aggregate genetic liability for a phenotype of interest (in this case, AUDIT scores and plasma EPA and DHA levels). Additional information on PRS is available in Choi et al. (2018) and Sugrue and Desikan (2019).

To derive PRS for the current study, we obtained publicly available summary statistics from the most well-powered and phenotypically appropriate GWAS identified through a literature search. Although a meta-analysis of two GWAS of SRE scores is available (Edwards et al., 2018) that study included the ALSPAC sample, rendering it unsuitable as a discovery dataset. We therefore used summary statistics from a GWAS of AUDIT (Babor et al., 2001) scores in the UK Biobank sample (Bycroft et al., 2018, Sanchez-Roige et al., 2019) disaggregated into the AUDIT-C and AUDIT-P to enable detection of potential differences in the association between genetic liability to each construct with ω–3 LC-PUFA levels. AUDIT-C consists of the first three AUDIT items and captures past-year alcohol consumption; AUDIT-P consists of the remaining 7 AUDIT items and captures past-year problematic use. Because the aim was to account for polygenic liability to alcohol consumption/problems rather than to dissect the impact of loci implicated at various levels of significance, only the inclusive $p<0.50$ threshold PRS was derived for inclusion as a predictor. Note that, while the Sanchez-Roige report includes both UK Biobank and 23andMe participants, only summary statistics for the former were used in the current analyses.

To account for genetic factors associated with ω–3 LC-PUFA levels, we downloaded summary statistics from meta-analyses of GWAS on plasma EPA and DHA levels, made available by the CHARGE Consortium (http://www.chargeconsortium.com/main/results) and reported by Lemaitre et al. (2011) The CHARGE study consisted of 8,866 participants of European ancestry, making it suitable as a discovery sample for ALSPAC. We chose to analyze the long-chain polyunsaturated fatty acids EPA and DHA because these long chain ω–3 fatty acids had been directly tested in animal models and had been shown to affect ethanol sensitivity (Wolstenholme et al., 2018) In addition, EPA and DHA are the main constituents of fish oil, a common dietary supplement.

Genotypes for ALSPAC participants are available for a fee to researchers will an approved project (see http://www.bristol.ac.uk/alspac/researchers/ for details). Genotyping and initial quality control of data were performed by ALSPAC analysts, unrelated to the current project. Genotyping in ALSPAC was performed on the Illumina HumanHap550 quad genome-wide SNP genotyping platform by 23andMe subcontracting the Wellcome Trust Sanger Institute, Cambridge, UK, and the Laboratory Corporation of America, Burlington, NC, USA. Individuals were excluded from analyses on the basis of excessive or minimal heterozygosity, gender mismatch, individual missingness (0.3%), cryptic relatedness as measured by identity by descent (genome-wide IBD 0.10%) and sample duplication. Individuals were assessed for population stratification using multi-dimensional scaling modelling seeded with HapMap Phase II release 22 reference populations. Individuals of non-European ancestry were removed from further analysis. ShapeIt v2 was used to impute to 1000 Genomes Phase 1, Version 3, Release December 2013. We excluded markers with MAF<0.01, deviation from HWE ($p < 5 \times 10\text{-}6$), genotyping rate <0.95, or INFO <0.80. Polygenic risk scores were derived using the --score and --dosage options in Plink 1.9 (www.cog-genomics.org/plink/1.9/) (Chang et al., 2015) for markers with p<0.5 in the discovery sample. This corresponding to the following numbers of SNPs contributing to the four PRS after pruning: AUDIT-C, 222,651; AUDIT-P, 222,118; DHA, 154,759; EPA, 156,352.

Statistical analyses

Analyses were conducted in R version 3.4.3 using the glm function, potentially using three stages of multivariable regressions. In the first stage, SRE scores were regressed onto ω–3 LC-PUFA levels, including biological sex (determined at birth) as a covariate. In the second stage, we added the main effects of ancestry-informative principal components as well as polygenic scores for alcohol

consumption, alcohol problems, DHA, and EPA. In the third stage, we added interaction terms between ω–3 LC-PUFA levels and any PRS variable for which a main effect ($p<0.05$) was observed for both the variables in the second stage, in order to test whether polygenic liability for the trait(s) in question moderated the ω–3 LC-PUFA→SRE association.

Although our primary research question focused on contemporaneous ω–3 LC-PUFA and SRE, we also considered the possibility that ω–3 LC-PUFA level would impact later SRE scores. We therefore ran regression models in which the age 15.5 ω–3 LC-PUFA measure was the predictor of interest for SRE at age 16.5 or 17.5. SRE scores, ω–3 LC-PUFA levels, and PRS scores were standardized prior to analysis for ease of interpretation.

Results

Descriptive statistics

Table 1 provides descriptive statistics for ω–3 LC-PUFA levels and SRE scores across waves. ω–3 LC-PUFA levels at ages 15.5 and 17.5 were correlated at $r = 0.49$ ($p < 0.0001$). Correlations within SRE scores were not calculated since only the first report was used for each individual. Both alcohol-related PRS were weakly positively correlated with the first reported SRE score ($r = 0.03$, $p =$ 0.04-0.06), indicating that higher genetic liability to alcohol consumption/problems was correlated with needing more standard units of alcohol to perceive its effects (i.e., lower alcohol sensitivity). DHA/EPA PRS were weakly positively correlated with ω–3 LC-PUFA levels ($r = 0.02$-0.05, $p = 0.01$-0.22). Correlations across ω–3 LC-PUFA levels and SRE scores ranged from $r = -0.18$ to $r = -0.05$ ($p <$ 0.01-0.38; **Figure**).

Model 1 regression results

Table 2 (top panel) provides results from Model 1, in which SRE scores were regressed onto ω–3 LC-PUFA levels and sex. Higher ω–3 LC-PUFA levels at age 15.5 were nominally associated ($p<0.05$) with lower SRE scores (i.e., higher initial alcohol sensitivity) at ages 15.5 and 17.5, but not at age 16.5. Age 17.5 ω–3 LC-PUFA levels were not associated with concurrent SRE scores.

Model 2 regression results

We next added PRS for alcohol consumption, alcohol problems, DHA levels, and EPA levels as potential predictors of initial alcohol sensitivity. Results are presented in **Table 2** (bottom panel). The associations between higher age 15.5 ω–3 LC-PUFA levels and lower SRE scores (i.e., higher alcohol sensitivity) at ages 15.5 and 17.5 persisted in these adjusted models. We also observed a negative association between AUDIT-P PRS and age 16.5 SRE scores. No other PRS was associated with SRE score at any age (all $p\geq0.05$). Because the only main effect of PRS was observed in a model in which no significant main effect of ω–3 LC-PUFA was observed, we did not test for an interaction with the PRS score.

Discussion

Prior research in model systems has demonstrated that ω–3 LC-PUFA levels may influence initial physiological sensitivity to alcohol, but associations between ω–3 LC-PUFA levels and sensitivity to alcohol in humans have not been reported, to our knowledge. Our analyses tested for an association between plasma ω–3 LC-PUFA levels and initial sensitivity to alcohol in a population-based sample of adolescents. We also assessed whether genetic factors underlying to alcohol outcomes (consumption or problems) or EPA/DHA levels further contributed to initial alcohol sensitivity. We

found that higher ω–3 LC-PUFA levels were associated with higher alcohol sensitivity in some, but not all, analyses. Polygenic scores exhibited little to no effect on the outcome, and moderation tests were not warranted. In conjunction with results from model systems, these findings extend our understanding of the relationship between ω–3 LC-PUFA levels and alcohol outcomes to include an individual's earliest experiences with the drug.

ω–3 LC-PUFA levels assessed at age 15.5 were associated with SRE scores both concurrently and two years later, but not with SRE scores in the intervening year. While the effect size at age 17.5 is nearly twice that at age 15.5, the standard error is much higher in the former and the corresponding significance value much weaker, and we observed no association between ω–3 LC-PUFA levels and age 16.5 initial sensitivity. We conducted four multivariable analyses, for which a corresponding conservative correction would require $p<0.0125$ to achieve statistical significance. This correction would further call into question the association between age 15.5 ω–3 LC-PUFA level and age 17.5 SRE; the within-age 15.5 association survives the correction. Our data, therefore, can most cautiously be interpreted as supporting a relationship between contemporaneous ω–3 LC-PUFA levels and initial alcohol sensitivity in early/mid-adolescence.

We observed a main effect of aggregate genetic liability toward alcohol problems — operationalized by scores on the problems subscale of the AUDIT — on age 16.5 SRE scores but in no other case. This association was not in the expected direction: Here, higher genetic liability to alcohol problems was associated with lower SRE scores (i.e., higher sensitivity). There were no main effects of polygenic liability for alcohol consumption, EPA levels, or DHA levels. Furthermore, in the absence of jointly observed main effects for both polygenic liability and ω–3 LC-PUFA levels, moderation tests were not warranted. Thus, although both the outcome and predictor of interest are

genetically influenced (Edwards et al., 2018, Lemaitre et al., 2011), it is unlikely that the degree of their phenotypic association in the ALSPAC sample is moderated by genetic factors. This observation is potentially pertinent to efforts to determine whether EPA and DHA supplementation could be used to modify the response to alcohol because, in the context of initial alcohol sensitivity in adolescence, response to ω–3 LC-PUFA supplementation is unlikely to depend heavily on one's underlying genetic vulnerability to alcohol problems.

It is important to note that the current approach does not determine whether the observed associations are causal in nature. In addition, because SRE scores are based on self-report, they may be imprecise and/or subject to recall bias. While ALSPAC participants are instructed to respond to the items used for the current study by recalling their first exposures to alcohol, these reports may be influenced by more recent drinking experiences. We therefore restricted our analyses to include only an individual's first report on the SRE items (though participants are administered the "first 5 or so" SRE items repeatedly across waves) in an effort to capture the report that was closest in time to the initial alcohol experiences.

We used a large, publicly available dataset of GWAS summary statistics for ω–3 LC-PUFA (from the CHARGE Consortium). While a meta-analysis of initial alcohol sensitivity GWAS has been conducted (Edwards et al., 2018), we were unable to use summary statistics from that study because genetic influences were driven by the ALSPAC sample, and therefore were not independent. We therefore elected to use summary statistics from the UK Biobank, as this is a statistically well-powered, population-based sample of the same ancestry as ALSPAC, and prior research indicates that PRS are more useful when used across similarly ascertained samples (Savage et al., 2018) We used summary statistics based on adult phenotypes, and given the dynamic nature of diet and alcohol use

296 across the life course, discovery samples closer in age to the ALSPAC sample will be valuable to
297 analyze in the future.

298 This work adds to the growing appreciation of the effects of dietary ω–3 LC-PUFA levels on
299 neurobiological outcomes, including substance use and psychopathology. Here we found that basal
300 levels of ω–3 LC-PUFA were associated with alcohol response in adolescence, though effect sizes
301 were small and may not be clinically significant. Previously, a small study of treatment-seeking
302 substance abusers found that low ω–3 LC-PUFA levels were associated with an increased risk of
303 relapse or study drop-out (Buydens-Branchey et al., 2009). Plasma ω–3 LC-PUFA levels were
304 positively correlated with alcohol consumption in non-alcoholic people in IMMIDIET study (di
305 Giuseppe et al., 2009). Together, these observations underscore the potential clinical implications of
306 ω–3 LC-PUFA levels on alcohol-related outcomes.

307 Our study is particularly significant because we examined a young population and looked at
308 initial responses to ethanol. In alcoholics, heavy drinking is associated with dysregulation of ω–3 LC-
309 PUFA levels, probably due in part to liver damage (Vatsalya et al., 2016). It may therefore be difficult
310 to distinguish between an effect of ω–3 LC-PUFA levels on ethanol responses, versus an effect of
311 alcohol abuse behavior on ω–3 levels. Our study of adolescents is less subject to that confound than
312 studies of adults with long-term histories of heavy drinking.

313 The mechanisms whereby ω–3 LC-PUFAs may influence low responses to alcohol are beyond
314 the scope of the current study but merit consideration. Both ω–3 LC-PUFAs and alcohol engage
315 neurotransmitter systems: Deficits in ω–3 LC-PUFAs adversely affect neuroinflammatory
316 mechanisms (Laye et al., 2018) which in turn impact stress hypothalamic–pituitary–adrenal (HPA)
317 axis sensitivity and other neurotransmitter systems (Levant, 2013). Effects of ω–3 LC-PUFA

deficiencies on dopaminergic neurons can be pronounced, especially in the ventral striatum (Healy-Stoffel and Levant, 2018). The dopaminergic system is of central relevance in alcohol responses (Schuckit, 2018); alcohol also has prominent simultaneous effects on gamma-aminobutyric acid (GABA), glutamate, opioid, serotonin, and acetylcholine systems, and on the HPA axis, each of which could contribute to alcohol sensitivity (Schuckit, 2018). Future studies could potentially investigate whether high versus low ω–3 LC-PUFA levels may contribute to differences in alcohol sensitivity via perturbation of specific neurotransmitter systems.

In summary, we report an association between plasma ω–3 LC-PUFA levels and initial alcohol sensitivity in a population-based cohort of adolescents, such that higher ω–3 LC-PUFA levels correspond to higher alcohol sensitivity. These findings, which primarily support a contemporaneous association in mid-adolescence, warrant follow-up in an independent sample. While our observational study cannot address causality, the results raise the possibility that dietary ω–3 LC-PUFA levels could reduce low initial responses to alcohol, which has been previously associated with the development of problematic alcohol outcomes (Schuckit, 1994). Our findings add to the growing body of literature suggesting important associations between low levels of ω–3 LC-PUFAs and increased risks for psychopathology.

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Figure caption

Correlations between unstandardized ω–3 long chain polyunsaturated fatty acid levels (in mmol/L; x-axis) and scores on the Self-Rating of the Effects of Alcohol (SRE; y-axis). Pearson correlations and corresponding p-values are presented, along with regression lines and shaded standard errors.

Table 1. Descriptive statistics for ω–3 LC-PUFA levels and Self-Rating of the Effects of Alcohol (SRE) scores.

	ω–3 LC-PUFA			SRE Score[1]	
	N	Mean (SD) mmol/L		N	Mean (SD)
Age 15.5					
Total	3361	0.28 (0.07)		3285	5.40 (2.95)
Girls	1749	0.30 (0.07)		1869	5.07 (2.83)
Boys	1612	0.26 (0.07)		1416	5.82 (3.05)
Age 16.5					
Total	n/a	n/a		1398	4.58 (2.43)
Girls	n/a	n/a		877	4.35 (2.32)
Boys	n/a	n/a		521	4.98 (2.56)
Age 17.5					
Total	3166	0.30 (0.08)		942	5.14 (2.62)
Girls	1647	0.31 (0.08)		446	4.65 (2.26)
Boys	1519	0.28 (0.07)		496	5.58 (2.84)

[1]Figures are restricted to participants' first SRE report, and represent UK standard drinks, one of which contains 8 grams of ethanol (a standard US drink has 14 grams of ethanol). The participants whose first SRE report is at age 15.5 do not overlap with those whose first SRE report is from a later age.

Table 2. Results from linear models where scores on the Self-Rating of the Effects of Alcohol (SRE) scale are regressed onto concurrent or prior ω–3 LC-PUFA levels and other potential predictors/covariates. In the interest of space, results for 10 ancestry-informative principal component covariates are not shown. Continuous variables were standardized prior to analysis, and beta coefficients represent the change in SRE associated with a unit change in ω–3 LC-PUFA.

	Age 15.5 ω–3 LC-PUFA and age 15.5 SRE			Age 15.5 ω–3 LC-PUFA and age 16.5 SRE			Age 15.5 ω–3 LC-PUFA and age 17.5 SRE			Age 17.5 ω–3 LC-PUFA and age 17.5 SRE	
	Beta (SE)	*p*-value		Beta (SE)	*p*-value		Beta (SE)	*p*-value		Beta (SE)	*p*-value
Model 1											
Sex[1]	-0.20 (0.05)	1.7e-5		-0.12 (0.11)	0.30		-0.51 (0.11)	1.0e-5		-0.33 (0.08)	9.6e-5
ω–3 LC-PUFA [2]	-0.07 (0.02)	9.7e-4		-0.03 (0.06)	0.58		-0.12 (0.06)	0.03		-0.01 (0.04)	0.73
Model 2											
Sex	-0.19 (0.05)	2.35e-4		-0.10 (0.13)	0.43		-0.36 (0.12)	2.77e-3		-0.22 (0.09)	0.03
ω–3 LC-PUFA	-0.08 (0.03)	1.67e-3		-0.01 (0.07)	0.88		-0.14 (0.06)	0.02		<0.01 (0.05)	0.94
AUDIT-C PRS	0.03 (0.03)	0.30		0.08 (0.07)	0.25		0.02 (0.06)	0.70		0.11 (0.05)	0.05
AUDIT-P PRS	<0.01 (0.03)	0.88		-0.17 (0.07)	0.02		0.11 (0.06)	0.09		0.04 (0.05)	0.41
DHA PRS	-0.01 (0.03)	0.66		0.06 (0.08)	0.42		0.01 (0.06)	0.83		0.02 (0.05)	0.64
EPA PRS	-0.02 (0.03)	0.56		-0.05 (0.07)	0.48		0.08 (0.06)	0.22		0.04 (0.05)	0.45

[1]Boys are the reference group

[2]mmol/L