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Association between genetically determined leptin and blood lipids considering alcohol consumption: a Mendelian randomisation study

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ABSTRACT

Objectives The objective of this study was to evaluate the association of genetically determined leptin with lipids.

Design We conducted a Mendelian randomisation study to assess a potential causal relationship between serum leptin and lipid levels. We also evaluated whether alcohol drinking modified the associations of genetically determined leptin with blood lipids.

Setting and participants 3860 participants of the Framingham Heart Study third generation cohort.

Results Both genetic risk scores (GRSs), the GRS generated using leptin loci independent of body mass index (BMI) and GRS generated using leptin loci dependent of BMI, were positively associated with log-transformed leptin (log-leptin). The BMI-independent leptin GRS was associated with log-transformed triglycerides (log-TG, β=−0.66, p=0.01), but not low-density lipoprotein cholesterol (LDL-C, p=0.99), high-density lipoprotein cholesterol (HDL-C, p=0.44) or total cholesterol (TC, p=0.49). Instrumental variable estimation showed that per unit increase in genetically determined log-leptin was associated with 0.55 (95% CI: 0.05 to 1.00) units decrease in log-TG. Besides significant association with log-TG (β=−0.59, p=0.009), the BMI-dependent GRS was nominally associated with HDL-C (β=−10.67, p=0.09) and TC (β=−28.05, p=0.08). When stratified by drinking status, the BMI-dependent GRS was associated with reduced levels of LDL-C (p=0.03), log-TG (p=0.004) and TC (p=0.003) among non-current drinkers only. Significant interactions between the BMI-dependent GRS and alcohol drinking were identified for LDL-C (p=0.03), log-TG (p=0.03) and TC (p=0.02).

Conclusion These findings together indicated that genetically determined leptin was negatively associated with lipid levels and the association may be modified by alcohol consumption.

Strengths and limitations of this study

- ► Population-based Mendelian randomisation studies may offer an opportunity to provide better evidence for the association of leptin with lipid metabolism in the adult population compared with observational epidemiology studies.
- ► The stringent quality control methods were used in measuring genotypes, phenotype and covariates in the current study to reduce measurement error and increase the statistical power.
- ► Pleiotropy effects of single-nucleotide polymorphisms included in the leptin genetic risk score (GRS) may confound the leptin GRS and lipids associations.
- ► Our analyses were restricted to individuals of European ancestry.

INTRODUCTION

Leptin is a key hormone that regulates appetite and food intake, body weight and energy balance.[1 2] Leptin is secreted primarily from the stomach, placenta and adipose tissue.[3] Biological studies have demonstrated that elevated leptin levels may play an important role in the pathogenesis of lipid accumulation.[4–9] As an active endocrine organ, the adipose tissue secretes leptin and plays a key role in immunometabolism.[10] Leptin can regulate both innate and adaptive immune responses[11 12] and subsequently regulate lipid profiles. Animal study demonstrated that hyperleptinaemia decreases the expression of sterol regulatory element binding protein 1 (SREBP-1c), a master regulator of lipid metabolism, in liver and adenovirus-induced hyperleptinaemia decreases triglyceride (TG) synthesis through SREBP-1c downregulation.[13] Meanwhile, SREBP-1c is involved in innate immune response in macrophages.[14] Therefore, it is rational to see immune connects with leptin in respect of lipid regulation. Case reports and case series have documented that leptin therapy can improve lipid profiles among patients with lipoatrophy or congenital leptin deficiency.[15–19] On contrary, in a cross-sectional survey of 12–16 years old high-school students, plasma leptin was positively associated with total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C),

low-density lipoprotein cholesterol (LDL-C) and TG.[20] Since observational epidemiologic studies cannot rule out all confounding effects, it is unclear whether such an association is causal. On the other hand, there are studies that demonstrate a neutral effect of leptin on blood lipid levels.[21] A small clinical trial that involved 17 patients with HIV-associated lipodystrophy suggested that leptin treatment did not improve fasting lipid kinetics.[21] Population-based Mendelian randomisation studies may offer an opportunity to provide better evidence for the effect of leptin on lipid metabolism in the adult population. Recently, a large-scale genome-wide association study (GWAS) meta-analysis identified five genomic loci associated with circulating leptin,[22] which provides an opportunity to conduct a Mendelian randomisation study to delineate the association between serum leptin and lipid levels. In addition, alcohol consumption has been shown to influence leptin secretion in both human and animal models.[23–36] In rodent models, leptin has been demonstrated to be increased[26–28] or decreased[29 30] after alcohol intake. Similarly, leptin levels in human was decreased,[32] increased[31 33] or even unchanged[34–36] after drinking. It is unclear whether alcohol consumption modifies the association of genetically determined leptin with lipid levels.[37 38]

Therefore, the objectives of the current study were to evaluate the relationship between genetically determined leptin and lipid levels and to explore whether the leptin–lipids associations could be modified by alcohol consumption among participants of the Framingham Heart Study (FHS) third generation cohort.

MATERIALS AND METHODS

Data sources and study participants

The FHS was designed to identify common factors or characteristics that contribute to cardiovascular disease (CVD) by tracking the development of CVD over a long period of time. Participants of the FHS were free from overt symptoms of CVD or stroke at baseline. Later on, the FHS was extended to including offspring and third generation of the original participants. A detailed description of the FHS third generation cohort has been outlined in previous publications.[39] Genotype and phenotype data of the FHS are catalogued on the database of genotype and phenotype (dbGaP) at the National Center for Biotechnology Information (NCBI). We have received approval to use the FHS data by the Institutional Review Boards at the University of Georgia and the NCBI. Serum leptin levels, genotypes, lipid levels and important covariates were available for 3860 (94.7%) participants of the third generation cohort at baseline in 2002–2005 (table 1). Those participants were included in the current analyses.

Genotyping and GRS

Genetic loci for circulating leptin levels have been reported in a large GWAS meta-analysis by Kilpeläinen and colleagues.[22] This study included 32 161 individuals of European ancestry and identified three single-nucleotide polymorphisms (SNPs), *GCKR* rs780093, *LEP* rs10487505 and *SLC32A1* rs6071166, that were robustly associated with body mass index (BMI) adjusted leptin at a genome-wide significance level ($p<5\times10^{-8}$). In addition, *GCKR* rs780093, *CCNL1* rs900400 and *FTO* rs8043757 were associated with circulating leptin without adjustment for BMI.[22] We assumed the additive genetic model for each SNP and constructed two genetic risk scores (GRSs) for leptin by combining leptin-increasing alleles for SNPs weighted by their corresponding effect sizes on logarithmically transformed leptin (log-leptin) as reported in the original GWAS meta-analysis.[22] The first score, GRS1, was generated using the three SNPs associated with BMI-adjusted leptin, and the second score, GRS2, using the three SNPs associated with leptin unadjusted for BMI.

Genome-wide SNPs were genotyped using Affymetrix and Illumina platforms in the FHS. The 1000 Genome genotype data for the FHS was already imputed and catalogued on the dbGaP. According to the document of the FHS,[40] before imputation, quality control removed SNPs with Hardy-Weinberg equilibrium $p<1\times10^{-6}$, missing rate>3%, minor allele frequency (MAF) <1%, missing physical position or cannot mapped to build 37 positions, Mendelian errors >1000 or duplicate. MACH software was used for genotype phasing, followed by imputation using MiniMac software.[41 42] Imputation results were summarised as dosage scores, which represent the expected numbers of copies of the coded allele for each SNP, ranging from 0 to 2. After imputation, SNPs with $r^2<0.30$, MAF <1% or Hardy-Weinberg equilibrium $p<1\times10^{-6}$ were removed. We retrieved genotypes of the SNPs for GRSs from the imputed data for all study participants (online supplementary tables S1 and S2).

Leptin and lipids measurement

In the FHS, blood samples were collected after overnight fasting and analysed following standard protocols.[43] Serum leptin levels were determined by ELISA method at R&D Systems using the Quantikine Human Leptin Immunoassay.[43] Leptin was logarithmically transformed for analyses in the current study so that the data distribution can meet the assumptions of linear regression models.

Fasting blood lipids, including TC, HDL-C and TG, were measured using automated enzymatic assays.[43] For participants taking lipid-lowering medications, TC was adjusted as TC/0.8.[44] After adjustment, LDL-C was calculated using the Friedewald formula.[45] The adjusted TC and LDL-C were used for analyses in the current study. TG were logarithmically transformed (log-TG) in the current study so that the data distribution can meet the assumptions of linear regression models.

Covariates

Demographic and health behavioural variables, including age, gender, education, smoking and drinking, were based on self-report. Education levels were categorised into 'no more than high school', 'some college' and 'bachelor's

Table 1 Characteristics of the study participants by GRS1* for log-leptin in FHS third generation cohort

Covariates	Overall (n=3860)	Quartiles of the leptin GRS Q1 (n=964)	Q2 (n=961)	Q3 (n=977)	Q4 (n=958)	P value
GRS, mean (SD)	0.07 (0.03)	0.03 (0.02)	0.06 (0.01)	0.08 (0.01)	0.11 (0.01)	
Age, years, mean (SD)	40.2 (8.9)	40.5 (8.7)	39.9 (8.8)	40.3 (9.1)	39.9 (8.8)	0.23
Male, N (%)	1808 (46.8)	453 (47.0)	437 (45.5)	460 (47.1)	458 (47.8)	0.89
Education levels, N (%)						
No more than high school	591 (15.4)	141 (14.7)	146 (15.3)	157 (16.1)	147 (15.4)	0.22
Some college	1213 (31.5)	306 (31.8)	287 (30.0)	313 (32.1)	307 (32.3)	
Bachelor's degree and above	2041 (53.1)	514 (53.5)	524 (54.8)	505 (51.8)	498 (52.3)	
Current smoker, N (%)	603 (15.6)	144 (15.0)	152 (15.8)	165 (16.9)	142 (14.8)	0.53
Current drinker, N (%)	3419 (89.1)	858 (89.4)	853 (89.1)	863 (88.9)	845 (89.0)	0.32
Physical activities index score, mean (SD)	37.5 (7.9)	37.8 (8.0)	37.3 (8.1)	37.4 (7.7)	37.4 (7.8)	0.51
BMI, kg/m^2, mean (SD)	26.9 (5.5)	26.9 (5.5)	26.7 (5.5)	26.9 (5.5)	27.1 (5.5)	0.94
Waist girth, inches, mean (SD)	36.6 (6.0)	36.7 (6.0)	36.3 (5.8)	36.7 (5.9)	36.8 (6.1)	0.70
Treated for lipids, N (%)	265 (6.9)	80 (8.3)	56 (5.8)	66 (6.8)	63 (6.6)	0.26
Treated for diabetes, N (%)	72 (1.9)	22 (2.3)	23 (2.4)	19 (1.9)	8 (0.8)	0.03
LDL-C, mg/dL, mean (SD)	111.7 (31.4)	112.1 (30.5)	111.5 (31.1)	111.9 (32.4)	111.3 (31.8)	0.94
HDL-C, mg/dL, mean (SD)	54.3 (16.1)	54.1 (15.4)	54.4 (15.9)	54.5 (16.2)	54.4 (16.7)	0.62
TG, mg/dL, median (IQR)	92.0 (65.0–138.0)	92.0 (65.0–142.0)	96.0 (66.0–140.0)	92.0 (65.0–137.0)	90.0 (63.0–134.0)	0.03†
TC, mg/dL, mean (SD)	188.8 (35.5)	189.1 (34.1)	188.9 (37.1)	189.5 (35.7)	187.9 (35.2)	0.64
Leptin, ng/dL, median (IQR)	12.5 (3.5–15.1)	6.7 (3.4–14.5)	7.2 (3.4–14.8)	7.7 (3.7–14.9)	7.7 (3.6–16.8)	0.02†
Log-leptin, mean (SD)	2.00 (1.1)	1.95 (1.0)	1.98 (1.1)	2.00 (1.0)	2.06 (1.1)	0.02
Age, sex, BMI and waist girth adjusted log-leptin, mean (SD)	2.0 (1.1)	2.0 (1.0)	2.0 (1.1)	2.0 (1.0)	2.1 (1.1)	0.00005

*GRS1 for leptin was generated by summing leptin increasing alleles of three SNPs adjusted for BMI, weighted by their corresponding effect sizes reported by Kilpeläinen *et al*.
†Log-leptin and TG were used to calculate the p values.
BMI, body mass index; GRS, genetic risk score;HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; Log-leptin, logarithmically transformed leptin; SNPs, single-nucleotide polymorphisms; TC, total cholesterol; TG, triglycerides.

degree or above'. Smoking was categorised into 'current smoker' or 'non-current smoker' and drinking status into 'current drinker' and 'non-current drinker'. Physical activity was measured with the physical activity index composite score, which was calculated by summing the number of hours spent in each activity intensity level weighted by their corresponding weight factor derived from the estimated oxygen consumption requirement for each intensity level.[46] BMI was calculated as weight in kilograms divided by the square of height in metres. Waist circumference was measured to next lower 1/4 inch by regional anthropometry.

Statistical analysis

Weighted GRSs for leptin were calculated for each participant as the sum of the products of the participant's dosage scores for each SNP and the SNP's estimated effect size. Since obesity is highly associated with both leptin and blood lipids, our main focus was on GRS1, the score generated using loci associated with leptin independent of BMI. The GRS1 for participants was then categorised into quartiles. Means and SD for continuous and frequencies and percentages for categorical characteristics at baseline were calculated for each quartile of the GRS1. P values for linear trends in those variables across quartiles of the GRS1 were estimated.

Three multivariate linear regression models were used to assess associations between log-leptin and lipids, leptin GRS and log-leptin and the leptin GRS and lipids, respectively. All models were adjusted for age, sex, BMI and waist circumference. To test robustness of the leptin GRS and lipids associations, we additionally controlled for education, smoking, drinking and physical activity index score in the fully adjusted models. To explore whether associations between the leptin GRS and lipids levels were modified by alcohol consumption, we performed stratified analyses by drinking status. In each stratum of the drinking status, we tested associations between leptin GRS and lipids by adjusting for age, sex, BMI and waist circumference in the base model and additionally adjusting for education, smoking and physical activity in the full model. Interactions between the leptin GRS and alcohol consumption were tested among the overall participants by adding drinking and the interaction term, GRS×drinking, to the models. All the above analyses were done for GRS1 and GRS2 separately. We quantified the strength of the causal association of leptin with lipids using the instrumental variable estimator.[47] The estimator was calculated as the ratio of the coefficient for leptin GRS and lipids association to the coefficient for the leptin GRS and log-leptin association from the base models.

To rule out the effect of lipid-lowering medications, sensitivity analyses were performed among those not taking lipid medication. To rule out the effect of both diabetes and lipid-lowering medications, sensitivity analyses were performed among those not taking lipid-lowering or glucose-lowering medications. All analyses were performed using SAS software (V.9.4; SAS Institute). Two-sided p values were provided, and p<0.05 was considered significant.

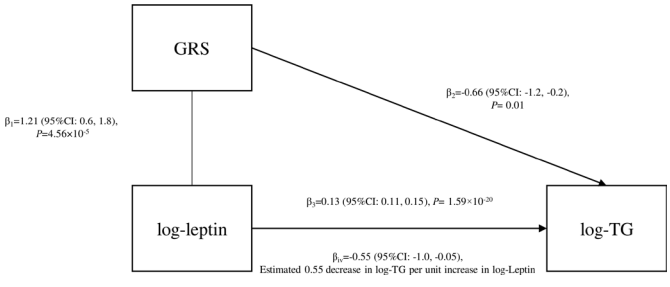

Figure 1 The relationship between log-leptin, GRS1 for leptin and log-TG in FHS the third generation cohort. FHS, Framingham Heart Study; GRS, genetic risk score; log-TG, logarithmically transformed triglycerides.

Participant and public involvement

Neither patients or public were directly involved in the development, design or recruitment of the study. Results will not be disseminated directly to study participants.

RESULTS

Characteristics of the study participants are presented in table 1. Participants were on average 40.2 years old at baseline. There were slightly more females (53.2%), and only 15.4% had less than a high-school education. The majority (89.1%) of the participants were current drinkers, and 15.6% were current smokers. Participants were on average over weighted, with a mean BMI of 26.9 kg/m^2 and mean waist girth of 36.6 inches. About 6.9% of the participants were treated for dyslipidemia, and 1.9% were treated for diabetes. The BMI-independent leptin GRS1 was not associated with age (p=0.23), sex (p=0.89), education (p=0.22), smoking (p=0.53), drinking (p=0.32), BMI (p=0.94), waist circumference (p=0.70), lipid-lowering medication usage (p=0.26) or the physical activity index score (p=0.51), but with diabetes-lowering medication usage (p=0.03). As expected, the GRS1 was positively associated with age, sex, BMI and waist circumference-adjusted log-leptin ($p=4.56\times10^{-5}$).

BMI-independent leptin GRS1 and blood lipids

After controlling for age, sex, BMI and waist circumference, log-leptin was positively associated with TC (β=8.56, $p=6.35\times10^{-18}$), LDL-C (β=6.46, $p=1.85\times10^{-13}$) and log-TG (β=0.13, $p=1.59\times10^{-20}$), but was not associated with HDL-C (β=−0.62, p=0.11, figure 1 and online supplementary figure S1). Per unit increase in the leptin GRS1 was associated with a 1.21 unit increase in the age, sex, BMI and waist circumference adjusted log-leptin ($p=4.56\times10^{-5}$). The leptin GRS1 was inversely associated with age, sex, BMI and waist circumference-adjusted log-TG (β=−0.66, p=0.01, figure 1). When further adjusting for education, smoking, drinking and physical activity, the GRS1 and log-TG association was still significant (β=−0.69, p=0.008, table 2). Instrumental variable estimation indicated that

Table 2 Association of BMI-independent leptin GRS1* with baseline lipids among overall, drinking and non-drinking participants of the FHS third generation cohort, respectively

	Age, sex, BMI, waist circumference adjusted model			Fully adjusted model‡		
	Beta (SE)	P value	$P_{interaction}$ †	Beta (SE)	P value	$P_{interaction}$ §
HDL-C						
Overall	5.42 (7.10)	0.44	0.74	7.79 (7.11)	0.27	0.71
Not current drinkers	20.42 (18.29)	0.26		22.22 (18.76)	0.24	
Current drinkers	6.00 (7.61)	0.43		7.02 (7.64)	0.36	
LDL-C						
Overall	−0.11 (16.09)	0.99	0.79	−1.09 (16.24)	0.95	0.93
Not current drinkers	3.37 (48.63)	0.94		−4.18 (50.10)	0.93	
Current drinkers	−0.14 (17.10)	0.99		−1.80 (17.18)	0.92	
Log-TG						
Overall	−0.66 (0.26)	0.01	0.31	−0.69 (0.26)	0.008	0.32
Not current drinkers	−1.41 (0.80)	0.08		−1.32 (0.82)	0.11	
Current drinkers	−0.58 (0.27)	0.04		−0.61 (0.27)	0.03	
Total cholesterol						
Overall	−12.50 (18.21)	0.49	0.96	−12.58 (18.31)	0.49	0.86
Not current drinkers	−15.20 (54.11)	0.78		−19.13 (55.66)	0.73	
Current drinkers	−10.20 (19.37)	0.60		−11.43 (19.42)	0.56	

*GRS1 for leptin was generated by summing leptin increasing alleles of three SNPs adjusted for BMI, weighted by their corresponding effect sizes reported by Kilpelainen *et al*.
†Assessed by adding an interaction term, drinking×GRS along with the drinking variable to the age, sex, BMI and waist circumference adjusted model among the overall participants.
‡Adjusted for age, sex, education, smoking, drinking, BMI, waist circumference and physical activity among the overall sample.
§Assessed by adding an interaction term, drinking×GRS to the fully adjusted model among the overall participants.
BMI, body mass index; FHS, Framingham Heart Study; GRS, genetic risk score; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; log-TG, logarithmically transformed triglycerides; SE, standard error; SNPs, single-nucleotide polymorphisms.

log-TG levels decreased by 0.55 (95% CI: 0.05, 1.00, p=0.02) per unit increase of genetically determined log-leptin level (figure 1). The leptin GRS1 was inversely associated with TC (β=−12.50, p=0.49) and LDL-C (β=−0.11, p=0.99) and positively associated with HDL-C (β=5.42, p=0.44), however, the correlations were not significant. The GRS1 and blood lipids associations were not modified by drinking status (table 2).

BMI-dependent leptin GRS2 and blood lipids

As expected, the BMI-dependent leptin GRS2 was not associated with any covariate except for the BMI (p=0.02) and waist circumference (p=0.03). In the analyses controlling for age, sex, BMI and waist circumference, the GRS2 was significantly associated with lower levels of log-TG (p=0.009) and nominally associated with lower levels of HDL-C (p=0.09) and TC (p=0.08, online supplementary figure S2). When stratified by drinking status, the leptin GRS2 was negatively associated with LDL-C (β=−92.51, p=0.03), log-TG (β=−2.07, p=0.004) and TC (β=−144.68, p=0.003) only among non-current drinkers (table 3). When further adjusting for education, smoking, drinking and physical actvity, those associations persisted (table 3). Furthermore, significant interactions between leptin GRS2 and alcohol drinking were identified for LDL-C (p=0.03), log-TG (p=0.03) and TC (p=0.02, table 3).

When restricting to participants not taking lipid-lowering medication and those not taking lipid- or glucose-lowering medications, respectively, the associations of GRS1 and GRS2 with blood lipids were similar to those as shown above (online supplementary tables S3–S6).

DISCUSSION

To the best of our knowledge, the current study is the first Mendelian randomisation analysis on leptin and blood lipids. We provide robust evidence to support a potentially causal relation between leptin and reduced levels of TG among a majority of overweight and obese population of European ancestry. Furthermore, we demonstrated that alcohol consumption modified the association of BMI-dependent GRS2 with lipids in that genetically determined leptin levels were inversely associated with LDL-C, log-TG and TC, but only among individuals who were not current drinkers.

Table 3 Association of BMI-dependent leptin GRS2* with baseline lipids among overall, drinking and non-drinking participants of the FHS third generation cohort, respectively

	Age, sex, BMI, waistadjusted model			Fully adjusted model‡		
	Beta (SE)	P value	$P_{interaction}$ †	Beta (SE)	P value	$P_{interaction}$ §
HDL-C						
Overall	−10.67 (6.20)	0.09	0.56	−10.98 (6.22)	0.08	0.52
Not current drinkers	−0.69 (16.31)	0.97		0.82 (16.55)	0.96	
Current drinkers	−12.15 (6.64)	0.07		−11.94 (6.68)	0.07	
LDL-C						
Overall	−2.11 (14.05)	0.88	0.03	−2.81 (14.21)	0.84	0.02
Not current drinkers	−92.51 (43.02)	0.03		−101.15 (43.78)	0.02	
Current drinkers	9.21 (14.91)	0.54		7.89 (15.02)	0.60	
Log-TG						
Overall	−0.59 (0.23)	0.009	0.03	−0.59 (0.23)	0.01	0.03
Not current drinkers	−2.07 (0.71)	0.004		−2.03 (0.72)	0.005	
Current drinkers	−0.40 (0.24)	0.09		−0.42 (0.24)	0.08	
Total cholesterol						
Overall	−28.05 (15.91)	0.08	0.02	−28.74 (16.02)	0.07	0.01
Not current drinkers	−144.68 (47.61)	0.003		−151.32 (48.37)	0.002	
Current drinkers	−13.19 (16.90)	0.44		−14.67 (16.98)	0.39	

*GRS2 for leptin was generated by summing leptin increasing alleles of three SNPs unadjusted for BMI, weighted by their corresponding effect sizes reported by Kilpelainen *et al.*
†Assessed by adding an interaction term, drinking×GRS along with the drinking variable to the age, sex, BMI and waist-adjusted model among the overall participants.
‡Adjusted for age, sex, education, smoking, drinking, BMI, waist and physical activity among the overall sample.
§Assessed by adding an interaction term, drinking×GRS to the fully adjusted model among the overall participants.
BMI, body mass index; FHS, Framingham Heart Study; GRS, genetic risk score; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; log-TG, logarithmically transformed triglycerides; SE, standard error.

Both the BMI dependent-GRS and independent-GRS were associated with lower level of log-TG in the current study. Inconsistent associations between leptin and blood lipids have been observed in previous studies. In a small study of 80 postmenopausal women, serum leptin was positively associated with HDL-C, TG and TC, and inversely associated with LDL-C.[48] Another study conducted with 294 healthy school children reported that leptin was only associated with increased TG.[49] However, a study of 476 residents from Cameroon reported a positive correlation between leptin, LDL-C and TC, and a positive association between leptin and TC, but no association between leptin and HDL-C or TG.[50] In a more recent study of 134 physically active postmenopausal women, no significant correlation was detected for leptin and blood lipids.[51] The divergent results of previous studies make it impossible to infer a relationship between leptin and blood lipids. Possible reasons for the divergent findings include varying sample sizes, failure to account for residual and unmeasured confounding and the genetic background of the study population. Through Mendelian randomisation analyses, we demonstrated that genetically determined leptin was inversely associated with log-TG. It is well known that alleles, such as risk alleles for leptin, are randomly assigned at meiosis and therefore, are independent of non-genetic confounders. The association between leptin GRS and log-TG in the current study was less prone to confounding. This also highlights the importance of using Mendelian randomisation to delineate causal relationships. Our finding is further supported by previous physiologic studies, among which, leptin was demonstrated to inhibit lipogenesis, stimulate lipolysis and reduce TG uptake.[52] However, the association of HDL-C and TC was only nominally significant with BMI-dependent GRS2 in the current study. It could be due to lack of statistical power or existing interaction of leptin and drinking. Therefore, we cannot rule out causal relationships between leptin and those lipid measures. Future large-scale Mendelian randomisation studies are warranted to evaluate associations of leptin GRS with HDL-C, LDL-C, and TC.

The BMI-independent GRS1 was only associated with log-TG, while the BMI-dependent GRS2 was also in nominal associations with HDL-C and TC. In addition, alcohol drinking modified the GRS2–lipids associations but not the GRS1–lipids associations. This indicated that the role of leptin in blood lipids regulation may be through multiple mechanisms. The BMI-dependent

GRS2 was inversely associated with LDL-C, log-TG and TC only among non-current drinkers, but not among current drinkers. Although future studies are warranted to confirm these interactions, previous physiological studies may provide a reasonable explanation. Singh and colleagues demonstrated that the increased expression of caveolin-1 impairs leptin signalling and attenuates leptin-dependent effect to prevent lipid accumulation in human white preadipocytes.[53] Meanwhile, caveolin-1 can be increased by alcohol drinking.[54]

Our study represents the first Mendelian randomisation analyses for leptin and blood lipids in a population of European ancestry. A major strength of this study is the stringent quality control methods used in measuring genotypes, phenotype and covariates in the FHS third generation cohort. Those methods can reduce measurement error and increase the statistical power needed to identify associations between leptin GRS and lipids. We also identify some limitations. First, pleiotropy effects of SNPs included in the leptin GRS may confound the leptin GRS and lipids associations. It is possible that our results may represent a shared genetic basis between leptin and lipids rather than a causal relationship. Second, we may not have sufficient power to detect associations between genetically determined leptin levels and LDL-C, HDL-C and TC. Larger Mendelian randomisation studies are warranted to evaluate associations between leptin and LDL-C, HDL-C and TC. Third, we did not control for total energy intake in our analyses because food frequency questionnaire survey was not conducted in the third generation cohort at baseline when leptin was measured. However, leptin combines with receptors in the hypothalamus to reduce appetite and increase energy expenditure. Therefore, total energy intake is in the pathway from leptin to lipids metabolism and may not meet the criteria of being a confounder. Forth, the type of alcohol consumed for current drinker was not measured and cannot be considered in the current analyses. It is possible that the alcohol consumed in the studied population is mainly wine and/or beers, which contains high level of resveratrol and phytochemical. The two chemicals may benefit lipid metabolism.[55 56] However, the two chemicals do not share similar genetic profile with leptin, and consequently, they should not be correlated with leptin and cannot affect the associations between leptin GRS and blood lipids. Fifth, genetically determined ratio of leptin to leptin receptor may be a better measure to study the role of leptin in lipid metabolism. However, we could not find a genome-wide study on the ratio of leptin to leptin receptor; therefore, a GRS on the ratio cannot be calculated. Future genome-wide studies on the ratio of leptin to leptin receptor are warranted. Finally, our analyses were restricted to individuals of European ancestry. Our findings may not be generalisable to populations of other ancestries.

In summary, the present study provided robust evidence for a potential causal effect of leptin on reduced TG. In addition, genetically determined leptin may regulate blood lipids through different mechanisms, and the association between leptin and lipid metabolism may be modified by alcohol consumption.

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