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BMJ Open

The effect of genetically determined leptin on blood lipids considering alcohol consumption

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3 **1 The effect of genetically determined leptin on blood lipids considering alcohol consumption**
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41 **16 Word count: 2,873**
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50 Abbreviations: CVD, cardiovascular disease; dbGaP, the database of genotype and phenotype; FHS, the
51 Framingham Heart Study; GRS, genetic risk score; GWAS, genome-wide association studies; HDL-C,
52 high density lipoprotein cholesterol; LDL-C, low density lipoprotein cholesterol; log-leptin,
53 logarithmically transformed leptin; log-TG, logarithmically transformed TG; TC, total cholesterol; TG,
54 triglycerides
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Abstract

Objectives: We evaluated the effect of genetically determined leptin on lipids using baseline data for 3,860 participants of the Framingham Heart Study 3rd Generation cohort.

Material and methods: Two genetic risk scores (GRSs) were generated using leptin loci independent and dependent of body mass index (BMI), respectively. Associations between leptin GRSs and leptin levels, leptin and lipid levels, and the leptin GRSs and lipid levels were assessed by multivariate linear regression models. Interactions between the GRSs and alcohol consumption were also evaluated in the models.

Results: Both GRSs were positively associated with log transformed leptin (log-leptin). The BMI independent leptin GRS was associated with log transformed triglycerides (log-TG) ($\beta=-0.66$, $p=0.01$), 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% confidence interval: 0.05-1.00) units decrease in log-TG. Besides significant association with log-TG ($\beta=-0.59$, $p=0.009$), the BMI dependent GRS was nominally associated with HDL-C ($\beta=-10.67$, $p=0.09$) and TC ($\beta=-28.05$, $p=0.08$). 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 ($p=0.03$), TG ($p=0.03$), and TC ($p=0.02$).

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3 1 **Conclusion:** These findings together indicated that genetically determined leptin reduced lipid
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5 2 levels and the effect may be modified by alcohol consumption.
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9 3 **Keywords:** leptin, lipids, alcohol consumption, genetic risk score
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12 4 **Strengths and limitations of this study:**
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- 15 5 • Population-based Mendelian randomization studies may offer an opportunity to provide
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17 6 better evidence for the effect of leptin on lipid metabolism in the adult population
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19 7 compared with observational epidemiology studies.
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21 8 • The stringent quality control methods were used in measuring genotypes, phenotype, and
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23 9 covariates in the current study to reduce measurement error and increase the statistical
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25 10 power.
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27 11 • Pleiotropy effects of SNPs included in the leptin genetic risk score (GRS) may confound
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29 12 the leptin GRS and lipids associations.
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31 13 • Our analyses were restricted to individuals of European ancestry.
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Introduction

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Leptin is a key hormone that regulates appetite and food intake, body weight, and energy balance (Campfield et al., 1995, Halaas et al., 1995). Leptin is secreted primarily from the stomach, placenta, and adipose tissue (Zhang et al., 1994). Biological studies have demonstrated that elevated leptin levels may play an important role in the pathogenesis of lipid accumulation (Enser and Ashwell, 1983, Harris, 2014, Kosztaczky et al., 2007, Sainz et al., 2015, Selenscig et al., 2010, Wang et al., 1999). Case reports and case series have documented that leptin therapy can improve lipid profiles among patients with lipoatrophy or congenital leptin deficiency (Ebihara et al., 2007, Javor et al., 2005, Kamran et al., 2012, Park et al., 2007, Paz-Filho et al., 2015). 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 triglycerides (TG) (Wu et al., 2001). 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 (Sekhar et al., 2012). A small clinical trial that involved 17 patients with HIV-associated lipodystrophy suggested that leptin treatment did not improve fasting lipid kinetics (Sekhar et al., 2012). Population-based Mendelian randomization 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 (Kilpelainen et al., 2016), which provides an opportunity to conduct a Mendelian randomization study to delineate the association between serum leptin and lipids levels. In addition, alcohol consumption has been shown to influence leptin secretion in both human and animal models

1 (Beulens et al., 2008, Greco et al., 2000, Henriksen et al., 1999, Maddalozzo et al., 2009, Nicolas
2 et al., 2001, Otaka et al., 2007, Pravdova et al., 2009, Rojdmarm et al., 2001, Roth et al., 2003,
3 Santolaria et al., 2003, Slomiany and Slomiany, 2009, Tan et al., 2012, Voican et al., 2015, Yu et
4 al., 2010). In rodent models, leptin has been demonstrated to be increased (Pravdova et al.,
5 2009, Slomiany and Slomiany, 2009, Yu et al., 2010) or decreased (Maddalozzo et al., 2009, Tan
6 et al., 2012) after alcohol intake. Similarly, leptin levels in human was decreased (Santolaria et
7 al., 2003), increased (Henriksen et al., 1999, Nicolas et al., 2001), or even unchanged (Beulens et
8 al., 2008, Greco et al., 2000, Voican et al., 2015) after drinking. It is unclear whether alcohol
9 consumption modifies the effect of genetically determined leptin on lipid levels
10 (Balasubramanian and Nalini, 2006, Wannamethee et al., 2007).

11 Therefore, the objectives of the current study were to evaluate the relationship between
12 genetically determined leptin and lipid levels and to explore whether the leptin-lipids
13 associations could be modified by alcohol consumption among participants of the Framingham
14 Heart Study (FHS) 3rd generation cohort.

15 **Materials and Methods**

16 **Data Sources and Study Participants**

17 The FHS was designed to identify common factors or characteristics that contribute to
18 cardiovascular disease (CVD) by tracking the development of CVD over a long period of time.
19 Participants of the FHS were free from overt symptoms of CVD or stroke at baseline. Later on,
20 the FHS was extended to including offspring and third generation of the original participants. A
21 detailed description of the FHS 3rd generation cohort has been outlined in previous publications
22 (Splansky et al., 2007). Genotype and phenotype data of the FHS are cataloged on the database

1 of genotype and phenotype (dbGaP) at the National Center for Biotechnology Information
2 (NCBI). We have received approval to use the FHS data by the Institutional Review Boards at
3 the University of Georgia and the NCBI. Circulating leptin levels, genotypes, lipid levels, and
4 important covariates were available for 3,860 (94.7%) participants of the 3rd generation cohort at
5 baseline in 2002-2005 (**Table 1**). Those participants were included in the current analyses.

6 **Genotyping and Genetic Risk Score**

7 Genetic loci for circulating leptin levels have been reported in a large genome-wide
8 association studies (GWAS) meta-analysis by Kilpelainen and colleagues(Kilpelainen et al.,
9 2016). This study included 32,161 individuals of European ancestry and identified three single-
10 nucleotide polymorphisms (SNPs), *GCKR* rs780093, *LEP* rs10487505, and *SLC32A1* rs6071166,
11 that were robustly associated with body mass index (BMI) adjusted leptin at a genome-wide
12 significance level ($p < 5 \times 10^{-08}$). In addition, *GCKR* rs780093, *CCNLI* rs900400, and *FTO*
13 rs8043757 were associated with circulating leptin without adjustment for BMI(Kilpelainen et al.,
14 2016). We assumed the additive genetic model for each SNP and constructed two genetic risk
15 scores (GRSs) for leptin by combining leptin-increasing alleles for SNPs weighted by their
16 corresponding effect sizes on logarithmically transformed leptin (log-leptin) as reported in the
17 original GWAS meta-analysis(Kilpelainen et al., 2016). The first score, GRS1, was generated
18 using the three SNPs associated with BMI adjusted leptin, and the second score, GRS2, using the
19 three SNPs associated with leptin unadjusted for BMI.

20 Genome-wide SNPs were genotyped using Affymetrix and Illumina platforms in the
21 FHS. The 1000 Genome genotype data for the FHS was already imputed and cataloged on the
22 dbGaP. According to the document of the FHS (2010), before imputation, quality control

1 removed SNPs with a Hardy-Weinberg equilibrium $p < 1 \times 10^{-6}$, a missing rate $> 3.1\%$, a minor
2 allele frequency (MAF) $< 1\%$, a missing physical position or cannot mapped to build 37 positions,
3 Mendelian errors > 1000 , or duplicate SNPs. MACH software was used for genotype phasing,
4 followed by imputation using MiniMac software (Auton et al., 2015, Das et al., 2016).
5 Imputation results were summarized as dosage scores, which represent the expected numbers of
6 copies of the coded allele for each SNP, ranging from 0 to 2. After imputation, SNPs with
7 $r^2 < 0.30$, an MAF $< 1\%$, or a Hardy-Weinberg equilibrium $p < 1 \times 10^{-6}$ were removed. We retrieved
8 genotypes of the SNPs for GRSs from the imputed data for all study participants (**Supplemental**
9 **Table S1 and Supplemental Table S2**).

10 **Leptin and Lipids measurement**

11 In the FHS, blood samples were collected after overnight fasting and analyzed following
12 standard protocols (Andersson et al., 2015). Serum leptin levels were determined by enzyme-
13 linked immunosorbent assay (ELISA) method at R&D Systems using the Quantikine Human
14 Leptin Immunoassay (Andersson et al., 2015). Leptin was logarithmically transformed for
15 analyses in the current study.

16 Fasting blood lipids, including TC, HDL-C, and TG, were measured using automated
17 enzymatic assays (Andersson et al., 2015). For participants taking lipid-lowering medications,
18 TC was adjusted as TC/0.8 (Rao et al., 2017). After adjustment, LDL-C was calculated using the
19 Friedewald formula (Friedewald et al., 1972). The adjusted TC and LDL-C and logarithmically
20 transformed TG (log-TG) were used for analyses in the current study.

21 **Covariates**

1 Demographic and health behavioral variables, including age, gender, education, smoking,
2 and drinking, were based on self-report. Education levels were categorized into “no more than
3 high school,” “some college,” and “bachelor’s degree or above.” Smoking was categorized into
4 “current smoker” or “not a current smoker” and drinking status into “current drinker” and “not a
5 current drinker.” Physical activity was measured with the physical activity index composite
6 score, which was calculated by summing the number of hours spent in each activity intensity
7 level weighted by their corresponding weight factor derived from the estimated oxygen
8 consumption requirement for each intensity level (Kannel and Sorlie, 1979). BMI was calculated
9 as weight in kilograms divided by the square of height in meters. Waist circumference was
10 measured to next lower 1/4 inch by regional anthropometry.

11 **Statistical Analysis**

12 Weighted GRSs for leptin were calculated for each participant as the sum of the products
13 of the participant’s dosage scores for each SNP and the SNP’s estimated effect size. Since
14 obesity is highly associated with both leptin and blood lipids, our main focus was on GRS1, the
15 score generated using loci associated with leptin independent of BMI. The GRS1 for participants
16 was then categorized into quartiles. Means and standard deviations for continuous and
17 frequencies and percentages for categorical characteristics at baseline were calculated for each
18 quartile of the GRS1. *p* values for linear trends in those variables across quartiles of the GRS1
19 were estimated.

20 Three multivariate linear regression models were used to assess associations between log-
21 leptin and lipids, leptin GRS and log-leptin, and the leptin GRS and lipids, respectively. All
22 models were adjusted for age, sex, BMI, and waist circumference. To test robustness of the

1 leptin GRS and lipids associations, we additionally controlled for education, smoking, drinking,
2 and physical activity index score in the fully adjusted models. To explore whether associations
3 between the leptin GRS and lipids levels were modified by alcohol consumption, we performed
4 stratified analyses by drinking status. In each stratum of the drinking status, we tested
5 associations between leptin GRS and lipids by adjusting for age, sex, BMI, and waist
6 circumference in the base model and additionally adjusting for education, smoking, and physical
7 activity in the full model. Interactions between the leptin GRS and alcohol consumption were
8 tested among the overall participants by adding drinking and the interaction term, GRS×drinking,
9 to the models. All the above analyses were done for GRS1 and GRS2 separately. We quantified
10 the strength of the causal association of leptin with lipids using the instrumental variable
11 estimator (Palmer et al., 2011). The estimator was calculated as the ratio of the coefficient for
12 leptin GRS and lipids association to the coefficient for the leptin GRS and log-leptin association
13 from the base models.

14 To rule out the effect of lipid-lowering medications, sensitivity analyses were performed
15 among those not taking lipid medication. To rule out the effect of both diabetes and lipid-
16 lowering medications, sensitivity analyses were performed among those not taking lipid- or
17 glucose-lowering medications. All analyses were performed using SAS software (version 9.4;
18 SAS Institute Inc., Cary, North Carolina). Two-sided p values were provided, and $p < 0.05$ was
19 considered significant.

20 Results

21 Characteristics of the study participants are presented in **Table 1**. Participants were on
22 average 40.2 years old at baseline. There were slightly more females (53.2%), and only 15.4%

1 had less than a high school education. The majority (89.1%) of the participants were current
2 drinkers, and 15.6% were current smokers. Participants were on average over weighted, with a
3 mean BMI of 26.9 kg/m² and mean waist girth of 36.6 inches. About 6.9% of the participants
4 were treated for dyslipidemia, and 1.9% were treated for diabetes. The BMI independent leptin
5 GRS1 was not associated with age ($p=0.23$), sex ($p=0.89$), education ($p=0.22$), smoking
6 ($p=0.53$), drinking ($p=0.32$), BMI ($p=0.94$), waist circumference ($p=0.70$), lipid-lowering
7 medication usage ($p=0.26$), or the physical activity index score ($p=0.51$), but with diabetes-
8 lowering medication usage ($p=0.03$). As expected, the GRS1 was positively associated with age,
9 sex, BMI, and waist circumference adjusted log-leptin ($p=4.56\times 10^{-5}$).

10 **BMI independent leptin GRS1 and blood lipids**

11 After controlling for age, sex, BMI, and waist circumference, log-leptin was positively
12 associated with TC ($\beta=8.56$, $p=6.35\times 10^{-18}$), LDL-C ($\beta=6.46$, $p=1.85\times 10^{-13}$), and log-TG ($\beta=0.13$,
13 $p=1.59\times 10^{-20}$), but was not associated with HDL-C ($\beta=-0.62$, $p=0.11$) (**Figure 1 and**
14 **Supplemental Figure S1**). Per unit increase in the leptin GRS1 was associated with a 1.21-unit
15 increase in the age, sex, BMI, and waist circumference adjusted log-leptin ($p=4.56\times 10^{-5}$). The
16 leptin GRS1 was inversely associated with age, sex, BMI, and waist circumference adjusted log-
17 TG ($\beta=-0.66$, $p=0.01$) (**Figure 1**). When further adjusting for education, smoking, drinking, and
18 physical activity, the GRS1 and log-TG association was still significant ($\beta=-0.69$, $p=0.008$,
19 **Table 2**). Instrumental variable estimation indicated that log-TG levels decreased by 0.55 (95%
20 CI: 0.05, 1.00, $p=0.02$) per unit increase of genetically determined log-leptin level (**Figure 1**).
21 The leptin GRS1 was inversely associated with TC ($\beta=-12.50$, $p=0.49$) and LDL-C ($\beta=-0.11$,
22 $p=0.99$) and positively associated with HDL-C ($\beta=5.42$, $p=0.44$), however, the correlations were

1 not significant. The GRS1 and blood lipids associations were not modified by drinking status
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1 not significant. The GRS1 and blood lipids associations were not modified by drinking status
(Table 2).

3 BMI dependent leptin GRS2 and blood lipids

4 As expected, the BMI dependent leptin GRS2 was not associated with any covariate
5 except for the BMI ($p=0.02$) and waist circumference ($p=0.03$). In the analyses controlling for
6 age, sex, BMI, and waist circumference, the GRS2 was significantly associated with lower levels
7 of log-TG ($p=0.009$) and nominally associated with lower levels of HDL-C ($p=0.09$) and TC
8 ($p=0.08$) (Supplemental Figure S2). When stratified by drinking status, the leptin GRS2 was
9 negatively associated with LDL-C ($\beta=-92.51$, $p=0.03$), log-TG ($\beta=-2.07$, $p=0.004$), and TC ($\beta=-$
10 144.68 , $p=0.003$) only among non-current drinkers (Table 3). When further adjusting for
11 education, smoking, drinking, and physical activity, those associations persisted (Table 3).
12 Furthermore, significant interactions between leptin GRS2 and alcohol drinking were identified
13 for LDL-C ($p=0.03$), log-TG ($p=0.03$), and TC ($p=0.02$) (Table 3).

14 When restricting to participants not taking lipid-lowering medication and those not taking
15 lipid- or glucose-lowering medications, respectively, the associations of GRS1 and GRS2 with
16 blood lipids were similar to those as shown above (Supplemental Table S3, S4, S5 and S6).

17 Discussion

18 To the best of our knowledge, the current study is the first Mendelian randomization
19 analysis on leptin and blood lipids. We provide robust evidence to support a potentially causal
20 relation between leptin and reduced levels of triglycerides among a majority of overweight and
21 obese population of European ancestry. Furthermore, we demonstrated that alcohol consumption

1 modified the association of BMI dependent GRS2 with lipids in that genetically determined
2 leptin levels were inversely associated with LDL-C, log-TG, and TC, but only among individuals
3 who were not current drinkers.

4 Both the BMI dependent- and independent- GRSs were associated with lower level of
5 log-TG in the current study. Inconsistent associations between leptin and blood lipids have been
6 observed in previous studies. In a small study of 80 postmenopausal women, serum leptin was
7 positively associated with HDL-C, TG, and TC, and inversely associated with LDL-C (Jaleel et
8 al., 2006). Another study conducted with 294 healthy school children reported that leptin was
9 only associated with increased TG (Kavazarakis et al., 2001). However, a study of 476 residents
10 from Cameroon reported a positive correlation between leptin, LDL-C, and TC, and a positive
11 association between leptin and TC, but no association between leptin and HDL-C or TG (Ayina
12 et al., 2016). In a more recent study of 134 physically active postmenopausal women, no
13 significant correlation was detected for leptin and blood lipids (Jürimäe et al., 2010). The
14 divergent results of previous studies make it impossible to infer a relationship between leptin and
15 blood lipids. Possible reasons for the divergent findings include varying sample sizes, failure to
16 account for residual and unmeasured confounding, and the genetic background of the study
17 population. Through Mendelian randomization analyses, we demonstrated that genetically
18 determined leptin was inversely associated with log-TG. It is well known that alleles, such as
19 risk alleles for leptin, are randomly assigned at meiosis and therefore, are independent of non-
20 genetic confounders. The association between leptin GRS and log-TG in the current study was
21 less prone to confounding. Our finding is further supported by previous physiologic studies,
22 among which, leptin was demonstrated to inhibit lipogenesis, stimulate lipolysis, and reduce
23 triglyceride uptake (Hynes and Jones, 2001). However, the association of HDL-C and TC were

1 only nominally significant with BMI dependent GRS2 in the current study. It could be due to
2 lack of statistical power or existing interaction of leptin and drinking. Therefore, we cannot rule
3 out causal relationships between leptin and those lipid measures. Future large-scale Mendelian
4 randomization studies are warranted to evaluate associations of leptin GRS with HDL-C, LDL-
5 C, and TC.

6 The BMI independent GRS1 was only associated with log-TG, while the BMI dependent
7 GRS2 was also in nominal associations with HDL-C and TC. In addition, alcohol drinking
8 modified the GRS2-lipids associations but not the GRS1-lipids associations. This indicated that
9 the role of leptin in blood lipids regulation may be through multiple mechanisms. The BMI
10 dependent GRS2 were inversely associated with LDL-C, log-TG, and TC only among non-
11 current drinkers, but not among current drinkers. Although future studies are warranted to
12 confirm these interactions, previous physiological studies may provide a reasonable explanation.
13 Singh and colleagues demonstrated that the increased expression of caveolin-1 impairs leptin
14 signaling and attenuates leptin-dependent effect to prevent lipid accumulation in human white
15 pre-adipocytes (Singh et al., 2012). Meanwhile, Caveolin-1 can be increased by alcohol drinking
16 (Gao et al., 2014).

17 Our study represents the first Mendelian randomization analyses for leptin and blood
18 lipids in a population of European ancestry. A major strength of this study is the stringent quality
19 control methods used in measuring genotypes, phenotype, and covariates in the FHS 3rd
20 Generation Cohort. Those methods can reduce measurement error and increase the statistical
21 power needed to identify associations between leptin GRS and lipids. We also identify some
22 limitations. First, pleiotropy effects of SNPs included in the leptin GRS may confound the leptin

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3 1 GRS and lipids associations. It is possible that our results may represent a shared genetic basis
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5 2 between leptin and lipids rather than a causal relationship. Second, we may not have sufficient
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7 3 power to detect associations between genetically determined leptin levels and LDL-C, HDL-C,
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9 4 and TC. Larger Mendelian randomization studies are warranted to evaluate associations between
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11 5 leptin and LDL-C, HDL-C, and TC. Finally, our analyses were restricted to individuals of
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13 6 European ancestry. Our findings may not be generalizable to populations of other ancestries.
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18 7 In summary, the present study provided robust evidence for a causal effect of leptin on
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20 8 reduced triglycerides. In addition, genetically determined leptin may regulate blood lipids
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22 9 through different mechanisms, and the effect of leptin on lipid metabolism may be modified by
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24 10 alcohol consumption.
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35 13 **Author Contributions**

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38 14 Conceptualization, Changwei Li, José Cordero, Jia-Sheng Wang, and Shengxu Li;
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40 15 Formal analysis, Luqi Shen and Ye Shen; Supervision, Changwei Li, José Cordero, Jia-Sheng
41
42 16 Wang, and Shengxu Li; Writing – original draft, Luqi Shen; Writing – review & editing,
43
44 17 Changwei Li, José Cordero, and Luqi Shen.
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48 18 **Conflict of interest**

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52 19 Conflicts of interest and disclosures: none.
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55 20 **Funding sources**

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Figure legends

Figure 1. The relationship between leptin, genetic risk score for leptin and triglycerides (TG) in Framingham Heart Study the 3rd Generation cohort.

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Table 1. Characteristics of the Study Participants by Genetic Risk Score 1 (GRS1)^a for logarithmically transformed Leptin in Framingham Heart Study 3rd Generation Cohort.

Covariates	Overall	Quartiles of the leptin GRS				P
	(n=3,860)	Q1 (n=964)	Q2 (n=961)	Q3 (n=977)	Q4 (n=958)	
Genetic risk score, 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 (%)						
<i>No more than high school</i>	591 (15.4)	141 (14.7)	146 (15.3)	157 (16.1)	147 (15.4)	
<i>Some college</i>	1213 (31.5)	306 (31.8)	287 (30.0)	313 (32.1)	307 (32.3)	0.22
<i>Bachelor's degree and above</i>	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
Log-leptin, mean (SD)	2.0 (1.1)	2.0 (1.0)	2.0 (1.1)	2.0 (1.0)	2.1 (1.1)	0.02
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

BMI=body mass index; Log-leptin=logarithmically transformed leptin; GRS=Genetic Risk Score; SD=standard deviation.

^a Genetic risk scores 1(GRS1) for leptin was generated by summing leptin increasing alleles of 3 SNPs adjusted for BMI, weighted by their corresponding effect sizes reported by Kilpelainen et al.

Table 2. Association of BMI independent Leptin GRS1^a with Baseline Lipids among Overall, Drinking, and Non-Drinking Participants of the Framingham Heart Study 3rd Generation Cohort, Respectively.

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

GRS=Genetic Risk Score; HDL-C =High-density lipoprotein cholesterol; LDL-C =Low-density lipoprotein cholesterol; log-TG=logarithmically transformed triglycerides; SE=standard error.

^a Genetic risk scores1 (GRS1) for leptin was generated by summing leptin increasing alleles of 3 SNPs adjusted for body mass index (BMI), weighted by their corresponding effect sizes reported by Kilpelainen et al;

^b 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;

^c Adjusted for age, sex, education, smoking, drinking, BMI, waist, and physical activity among the overall sample;

^d Assessed by adding an interaction term, drinking×GRS to the fully adjusted model among the overall participants.

Table 3. Association of BMI dependent Leptin GRS^{2a} with Baseline Lipids among Overall, Drinking, and Non-Drinking Participants of the Framingham Heart Study 3rd Generation Cohort, Respectively.

	Age, sex, BMI, waist adjusted model		$P_{\text{interaction}}^b$	Fully adjusted model ^c		$P_{\text{interaction}}^d$
	Beta(SE)	P		Beta(SE)	P	
HDL-C						
Overall	-10.67 (6.20)	0.09		-10.98 (6.22)	0.08	
Not current drinkers	-0.69 (16.31)	0.97	0.56	0.82 (16.55)	0.96	0.52
Current drinkers	-12.15 (6.64)	0.07		-11.94 (6.68)	0.07	
LDL-C						
Overall	-2.11 (14.05)	0.88		-2.81 (14.21)	0.84	
Not current drinkers	-92.51 (43.02)	0.03	0.03	-101.15 (43.78)	0.02	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		-0.59 (0.23)	0.01	
Not current drinkers	-2.07 (0.71)	0.004	0.03	-2.03 (0.72)	0.005	0.03
Current drinkers	-0.40 (0.24)	0.09		-0.42 (0.24)	0.08	
Total cholesterol						
Overall	-28.05 (15.91)	0.08		-28.74 (16.02)	0.07	
Not current drinkers	-144.68 (47.61)	0.003	0.02	-151.32 (48.37)	0.002	0.01
Current drinkers	-13.19 (16.90)	0.44		-14.67 (16.98)	0.39	

GRS=Genetic Risk Score; HDL-C =High-density lipoprotein cholesterol; LDL-C =Low-density lipoprotein cholesterol; log-TG=logarithmically transformed triglycerides; SE=standard error.

^a Genetic risk scores² (GRS²) for leptin was generated by summing leptin increasing alleles of 3 SNPs unadjusted for body mass index (BMI), weighted by their corresponding effect sizes reported by Kilpelainen et al;

^b 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;

^c Adjusted for age, sex, education, smoking, drinking, BMI, waist, and physical activity among the overall sample;

^d Assessed by adding an interaction term, drinking×GRS to the fully adjusted model among the overall participants.

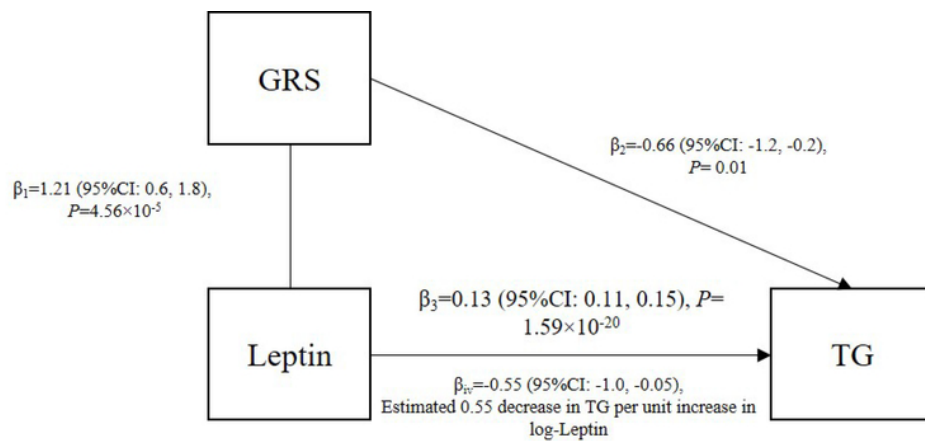


Figure 1

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Supplemental Table S1. Basic Information of three SNPs for BMI independent leptin GRS1^a reaching genome-wide significance ($P < 5 \times 10^{-8}$)

Chromosome		Coded	Non-coded	Nearest			
Position	rsID	Allele	Allele	R ²	Function	Gene	Population
2:27742603	rs780093	C	T	0.995	intron variant	GCKR	European American
7:127860163	rs10487505	G	C	0.989	intron variant	LEP	European American
20:37333012	rs6071166	C	A	0.973	intergenic	SLC32A1	European American

BMI=body mass index

^a Genetic risk scores 1 (GRS1) for leptin was generated by summing leptin increasing alleles of 3 SNPs adjusted for body mass index (BMI), weighted by their corresponding effect sizes reported by Kilpelainen et al.

Supplemental Table S2. Basic Information of three SNPs for BMI dependent leptin GRS2^a reaching genome-wide significance (P<5×10⁻⁸)

Chromosome		Coded	Non-coded	Nearest			
Position	rsID	Allele	Allele	R ²	Function	Gene	Population
2:27742603	rs780093	C	T	0.995	intron variant	GCKR	European American
3:156798775	rs900400	T	C	0.691	upstream variant 2KB	CCNL1	European American
16:53813450	rs8043757	A	T	0.999	intron variant	FTO	European American

BMI=body mass index

^a Genetic risk scores 2 (GRS2) for leptin was generated by summing leptin increasing alleles of 3 SNPs unadjusted for body mass index (BMI), weighted by their corresponding effect sizes reported by Kilpelainen et al.

Supplemental Table S3. Association of BMI independent Leptin GRS1^a with Baseline Lipids among Overall, Drinking, and Non-Drinking Participants without treating for Lipids of the Framingham Heart Study 3rd Generation Cohort, Respectively.

	Age, sex, BMI, waist adjusted model		<i>P</i> _{interaction} ^b	Fully adjusted model ^c		<i>P</i> _{interaction} ^d
	Beta(SE)	<i>P</i>		Beta(SE)	<i>P</i>	
HDL-C						
Overall	5.25 (7.44)	0.48		7.59 (7.47)	0.31	
Not current drinkers	18.83 (19.59)	0.34	0.86	20.43 (20.23)	0.31	0.83
Current drinkers	6.22 (7.97)	0.44		7.19 (8)	0.37	
LDL-C						
Overall	14.86 (16.06)	0.35		13.43 (16.2)	0.41	
Not current drinkers	36.1 (49.22)	0.46	0.51	27.18 (50.69)	0.59	0.61
Current drinkers	12.19 (17.05)	0.47		10.48 (17.11)	0.54	
log-TG						
Overall	-0.69 (0.27)	0.01		-0.72 (0.27)	0.007	
Not current drinkers	-1.72 (0.84)	0.04	0.20	-1.61 (0.86)	0.06	0.21
Current drinkers	-0.58 (0.28)	0.04		-0.6 (0.28)	0.03	

Total cholesterol

Overall	-0.09 (18.19)	1.00		-0.67 (18.26)	0.97	
Not current drinkers	3.78 (55.05)	0.95	0.94	-2.29 (56.42)	0.97	0.97
Current drinkers	0.98 (19.29)	0.96		-0.34 (19.32)	0.99	

BMI=body mass index; GRS=Genetic Risk Score; HDL-C=High-density lipoprotein cholesterol; LDL-C=Low-density lipoprotein cholesterol; log-TG=logarithmically transformed triglycerides; SE=standard error

^a Genetic risk scores 1 (GRS1) for leptin was generated by summing leptin increasing alleles of 3 SNPs adjusted for body mass index (BMI), weighted by their corresponding effect sizes reported by Kilpelainen et al;

^b 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;

^c Adjusted for age, sex, education, smoking, drinking, BMI, waist, and physical activity among the overall sample;

^d Assessed by adding an interaction term, drinking×GRS to the fully adjusted model among the overall participants.

Supplemental Table S4. Association of BMI dependent Leptin GRS2^a with Baseline Lipids among Overall, Drinking, and Non-Drinking Participants without treating for Lipids of the Framingham Heart Study 3rd Generation Cohort, Respectively.

	Age, sex, BMI, waist adjusted model			Fully adjusted model ^c		
	Beta(SE)	<i>P</i>	<i>P</i> _{interaction} ^b	Beta(SE)	<i>P</i>	<i>P</i> _{interaction} ^d
HDL-C						
Overall	-9.73 (6.50)	0.13		-9.66 (6.53)	0.14	
Not current drinkers	-2.61 (17.44)	0.88	0.72	-1.22 (17.77)	0.95	0.68
Current drinkers	-10.52 (6.94)	0.13		-10.19 (6.99)	0.15	
LDL-C						
Overall	-0.42 (14.02)	0.98		-1.56 (14.17)	0.91	
Not current drinkers	-83.19 (43.58)	0.06	0.07	-94.12 (44.22)	0.03	0.05
Current drinkers	9.71 (14.86)	0.51		7.61 (14.96)	0.61	
log-TG						
Overall	-0.63 (0.23)	0.007		-0.63 (0.23)	0.007	
Not current drinkers	-2.09 (0.74)	0.005	0.04	-2.00 (0.75)	0.008	0.05
Current drinkers	-0.45 (0.25)	0.07		-0.47 (0.25)	0.05	
Total cholesterol						

Overall	-25.28 (15.87)	0.11		-26.38 (15.97)	0.10	
Not current drinkers	-136.15 (48.44)	0.005	0.03	-144.29 (48.92)	0.003	0.02
Current drinkers	-10.96 (16.82)	0.51		-13.64 (16.90)	0.42	

BMI=body mass index; GRS=Genetic Risk Score; HDL-C=High-density lipoprotein cholesterol; LDL-C=Low-density lipoprotein cholesterol; log-TG=logarithmically transformed triglycerides; SE=standard error

^a Genetic risk scores 2 (GRS2) for leptin was generated by summing leptin increasing alleles of 3 SNPs unadjusted for body mass index (BMI), weighted by their corresponding effect sizes reported by Kilpelainen et al;

^b 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;

^c Adjusted for age, sex, education, smoking, drinking, BMI, waist, and physical activity among the overall sample;

^d Assessed by adding an interaction term, drinking×GRS to the fully adjusted model among the overall participants.

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Supplemental Table S5. Association of BMI independent Leptin GRS1^a with Baseline Lipids among Overall, Drinking, and Non-Drinking Participants without treating for Lipids and Diabetes of the Framingham Heart Study 3rd Generation Cohort, Respectively.

	Age, sex, BMI, waist adjusted model			Fully adjusted model ^c		
	Beta(SE)	P	P _{interaction} ^b	Beta(SE)	P	P _{interaction} ^d
HDL-C						
Overall	5.64 (7.47)	0.45		8.00 (7.49)	0.29	
Not current drinkers	20.89 (19.62)	0.29	0.72	22.51 (20.25)	0.27	0.69
Current drinkers	6.22 (7.98)	0.44		7.17 (8.02)	0.37	
LDL-C						
Overall	15.29 (16.11)	0.34		13.42 (16.27)	0.41	
Not current drinkers	33.51 (49.77)	0.50	0.58	24.63 (51.26)	0.63	0.68
Current drinkers	12.68 (17.09)	0.46		10.99 (17.16)	0.52	
log-TG						
Overall	-0.66 (0.27)	0.01		-0.69 (0.27)	0.01	
Not current drinkers	-1.60 (0.85)	0.06	0.23	-1.46 (0.86)	0.09	0.25
Current drinkers	-0.57 (0.28)	0.04		-0.59 (0.28)	0.04	

Total cholesterol

Overall	1.17 (18.24)	0.95		0.14 (18.33)	0.99	
Not current drinkers	6.19 (55.64)	0.91	0.93	1.04 (57.03)	0.99	0.99
Current drinkers	1.56 (19.35)	0.94		0.26 (19.38)	0.99	

BMI=body mass index; GRS=Genetic Risk Score; HDL-C =High-density lipoprotein cholesterol; LDL-C =Low-density lipoprotein cholesterol; log-TG=logarithmically transformed triglycerides; SE=standard error

^a Genetic risk scores 1 (GRS1) for leptin was generated by summing leptin increasing alleles of 3 SNPs adjusted for body mass index (BMI), weighted by their corresponding effect sizes reported by Kilpelainen et al;

^b 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;

^c Adjusted for age, sex, education, smoking, drinking, BMI, waist, and physical activity among the overall sample;

^d Assessed by adding an interaction term, drinking×GRS to the fully adjusted model among the overall participants.

Supplemental Table S6. Association of BMI dependent Leptin GRS2^a with Baseline Lipids among Overall, Drinking, and Non-Drinking Participants without treating for Lipids and Diabetes of the Framingham Heart Study 3rd Generation Cohort, respectively.

	Age, sex, BMI, waist adjusted model		<i>P</i> _{interaction^b}	Fully adjusted model ^c		<i>P</i> _{interaction^d}
	Beta(SE)	<i>P</i>		Beta(SE)	<i>P</i>	
HDL-C						
Overall	-10.39 (6.52)	0.11		-10.48 (6.55)	0.11	
Not current drinkers	-8.18 (17.54)	0.64	0.95	-7.77 (17.87)	0.66	0.92
Current drinkers	-10.58 (6.96)	0.13		-10.32 (7.01)	0.14	
LDL-C						
Overall	-1.58 (14.07)	0.91		-3.3 (14.22)	0.82	
Not current drinkers	-95.04 (44.19)	0.03	0.04	-105.66 (44.81)	0.02	0.03
Current drinkers	8.84 (14.90)	0.55		6.84 (15.00)	0.65	
log-TG						
Overall	-0.62 (0.23)	0.008		-0.62 (0.23)	0.008	
Not current drinkers	-2.00 (0.75)	0.008	0.06	-1.87 (0.76)	0.01	0.08

Current drinkers	-0.45 (0.25)	0.07		-0.48 (0.25)	0.05	
Total cholesterol						
Overall	-26.72 (15.92)	0.09		-28.67 (16.03)	0.07	
Not current drinkers	-150.52 (49.04)	0.002	0.01	-158.43 (49.51)	0.002	0.01
Current drinkers	-12.06 (16.87)	0.47		-14.69 (16.95)	0.39	

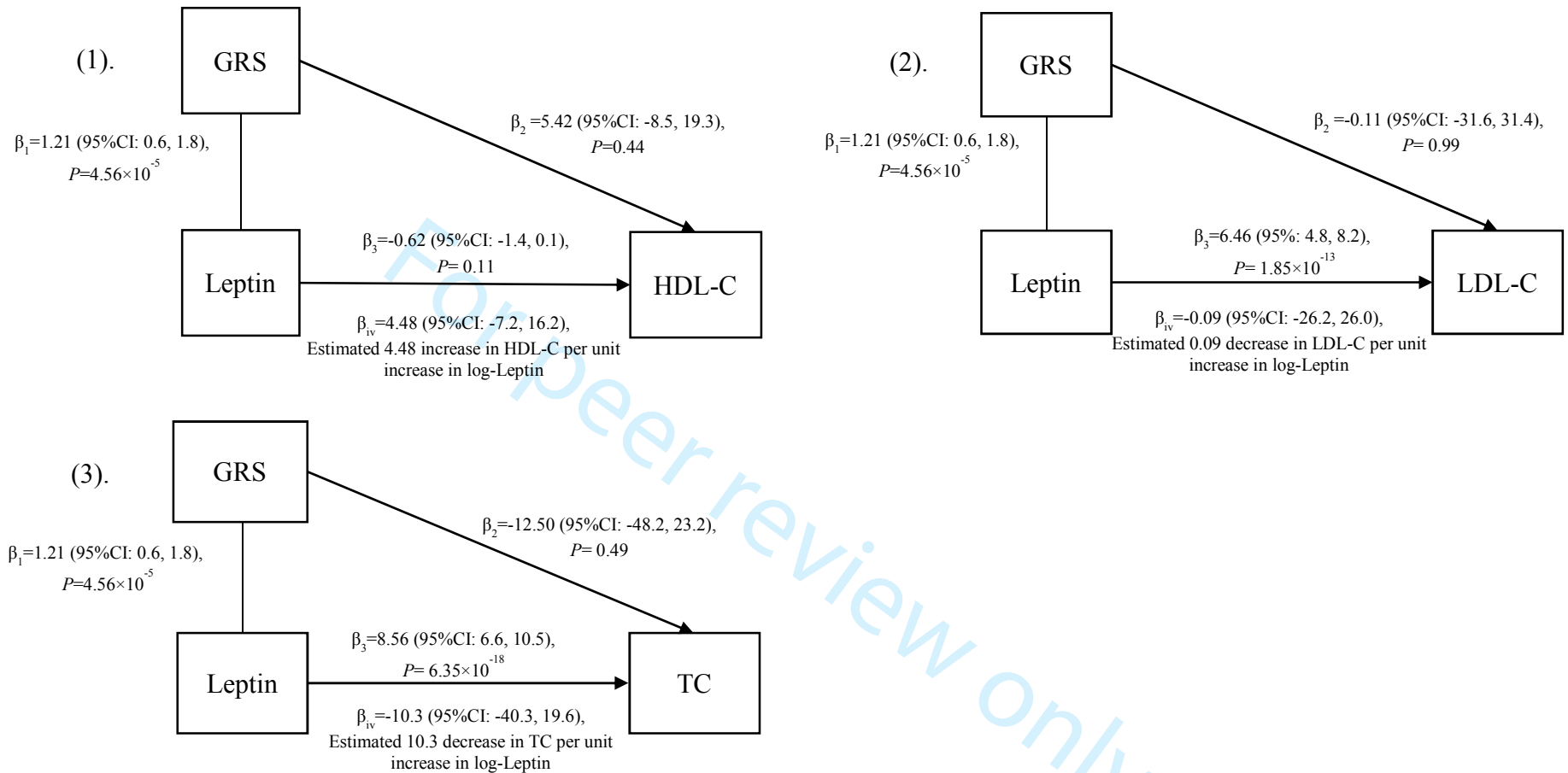
BMI=body mass index; GRS=Genetic Risk Score; HDL-C=High-density lipoprotein cholesterol; LDL-C=Low-density lipoprotein cholesterol; log-TG=logarithmically transformed triglycerides; SE=standard error

^a Genetic risk scores 2 (GRS2) for leptin was generated by summing leptin increasing alleles of 3 SNPs unadjusted for body mass index (BMI), weighted by their corresponding effect sizes reported by Kilpelainen et al;

^b 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;

^c Adjusted for age, sex, education, smoking, drinking, BMI, waist, and physical activity among the overall sample;

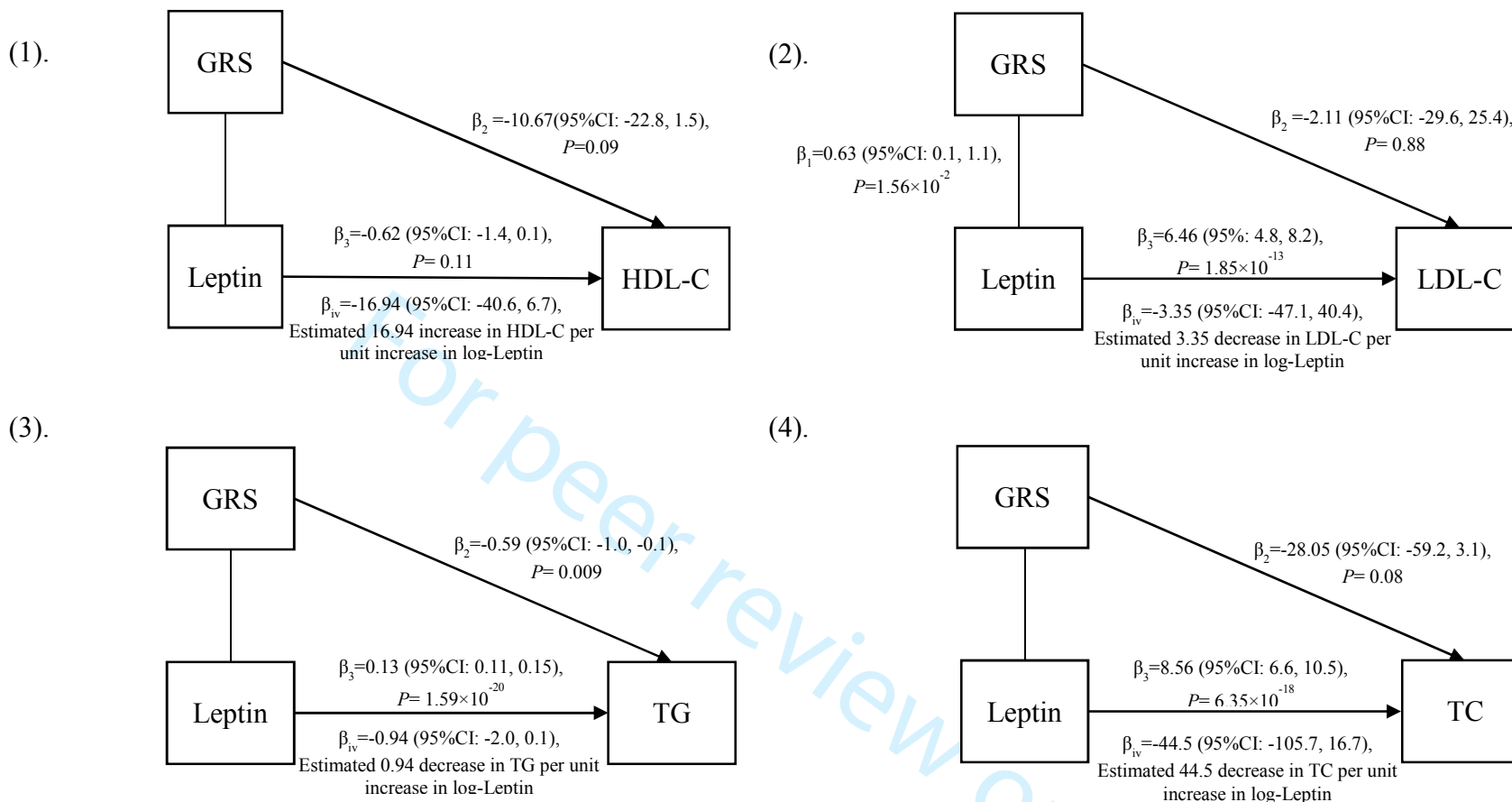
^d Assessed by adding an interaction term, drinking×GRS to the fully adjusted model among the overall participants.



Supplemental Figure S1. The relationship between Leptin, Genetic Risk Score 1 (GRS1) for Leptin and Lipids in Framingham

Heart Study the 3rd Generation cohort.

1. The relationship between leptin, genetic risk score for leptin and high density lipoprotein cholesterol (HDL-C)
2. The relationship between leptin, genetic risk score for leptin and low density lipoprotein cholesterol (LDL-C)
3. The relationship between leptin, genetic risk score for leptin and total cholesterol (TC)



Supplemental Figure S2. The relationship between Leptin, Genetic Risk Score 2 (GRS2) for Leptin and Lipids in Framingham Heart Study the 3rd Generation cohort.

1. The relationship between leptin, genetic risk score for leptin and high density lipoprotein cholesterol (HDL-C)
2. The relationship between leptin, genetic risk score for leptin and low density lipoprotein cholesterol (LDL-C)
3. The relationship between leptin, genetic risk score for leptin and triglycerides (TG)

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4. The relationship between leptin, genetic risk score for leptin and total cholesterol (TC)

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Reporting checklist for genetic association study.

Based on the STREGA guidelines.

Instructions to authors

Complete this checklist by entering the page numbers from your manuscript where readers will find each of the items listed below.

Your article may not currently address all the items on the checklist. Please modify your text to include the missing information. If you are certain that an item does not apply, please write "n/a" and provide a short explanation.

Upload your completed checklist as an extra file when you submit to a journal.

In your methods section, say that you used the STREGA reporting guidelines, and cite them as:

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		Reporting Item	Page Number
Title	#1a	Indicate the study's design with a commonly used term in the title or the abstract	1
Abstract	#1b	Provide in the abstract an informative and balanced summary of what was done and what was found	2
	#2	Explain the scientific background and rationale for the investigation being reported	4
	#3	State specific objectives, including any prespecified hypotheses. State if the study is the first report of a genetic association, a replication effort, or both.	5
	#4	Present key elements of study design early in the paper	5
	#5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	5-6

1	#6a	Cohort study – Give the eligibility criteria, and the sources and methods	5-6
2		of selection of participants. Describe methods of follow-up. Case-control	
3		study – Give the eligibility criteria, and the sources and methods of case	
4		ascertainment and control selection. Give the rationale for the choice of	
5		cases and controls. Cross-sectional study – Give the eligibility criteria,	
6		and the sources and methods of selection of participants. Give	
7		information on the criteria and methods for selection of subsets of	
8		participants from a larger study, when relevant.	
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14	#6b	Cohort study – For matched studies, give matching criteria and number	n/a
15		of exposed and unexposed. Case-control study – For matched studies,	
16		give matching criteria and the number of controls per case.	
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19	#7a	Clearly define all outcomes, exposures, predictors, potential	7-8
20		confounders, and effect modifiers. Give diagnostic criteria, if applicable	
21			
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23	#7b	Clearly define genetic exposures (genetic variants) using a widely-used	6-7
24		nomenclature system. Identify variables likely to be associated with	
25		population stratification (confounding by ethnic origin).	
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28	#8a	For each variable of interest give sources of data and details of methods	5-6
29		of assessment (measurement). Describe comparability of assessment	
30		methods if there is more than one group. Give information separately for	
31		for exposed and unexposed groups if applicable.	
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35	#8b	Describe laboratory methods, including source and storage of DNA,	6-7
36		genotyping methods and platforms (including the allele calling algorithm	
37		used, and its version), error rates and call rates. State the laboratory /	
38		centre where genotyping was done. Describe comparability of laboratory	
39		methods if there is more than one group. Specify whether genotypes	
40		were assigned using all of the data from the study simultaneously or in	
41		smaller batches.	
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46	#9a	Describe any efforts to address potential sources of bias	8-9
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48	#9b	Describe any efforts to address potential sources of bias	8-9
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51	#10	Explain how the study size was arrived at	5-6
52			
53	#11	Explain how quantitative variables were handled in the analyses. If	6
54		applicable, describe which groupings were chosen, and why. If	
55		applicable, describe how effects of treatment were dealt with.	
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59	#12a	Describe all statistical methods, including those used to control for	8-9
60		For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml	

1	confounding. State software version used and options (or settings)	
2	chosen.	
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4	#12b Describe any methods used to examine subgroups and interactions	9
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6	#12c Explain how missing data were addressed	9
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8	#12d If applicable, explain how loss to follow-up was addressed	n/a
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11	#12e Describe any sensitivity analyses	9
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13	#12f State whether Hardy-Weinberg equilibrium was considered and, if so,	7
14	how.	
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17	#12g Describe any methods used for inferring genotypes or haplotypes	7
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19	#12h Describe any methods used to assess or address population	7
20	stratification.	
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23	#12i Describe any methods used to address multiple comparisons or to	7
24	control risk of false positive findings.	
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27	#12j Describe any methods used to address and correct for relatedness	n/a
28	among subjects	
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31	#13a Report numbers of individuals at each stage of study—eg numbers	5-6
32	potentially eligible, examined for eligibility, confirmed eligible, included in	
33	the study, completing follow-up, and analysed. Give information	
34	separately for for exposed and unexposed groups if applicable. Report	
35	numbers of individuals in whom genotyping was attempted and numbers	
36	of individuals in whom genotyping was successful.	
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41	#13b Give reasons for non-participation at each stage	n/a
42		
43	#13c Consider use of a flow diagram	n/a
44		
45	#14a Give characteristics of study participants (eg demographic, clinical,	9-10
46	social) and information on exposures and potential confounders. Give	
47	information separately for exposed and unexposed groups if applicable.	
48	Consider giving information by genotype	
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52	#14b Indicate number of participants with missing data for each variable of	9-10
53	interest	
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56	#14c Cohort study – Summarize follow-up time, e.g. average and total	n/a
57	amount.	
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- 1 #15 Cohort study Report numbers of outcome events or summary measures 9/10
 2 over time. Give information separately for exposed and unexposed
 3 groups if applicable. Report outcomes (phenotypes) for each genotype
 4 category over time Case-control study – Report numbers in each
 5 exposure category, or summary measures of exposure. Give information
 6 separately for cases and controls . Report numbers in each genotype
 7 category. Cross-sectional study – Report numbers of outcome events or
 8 summary measures. Give information separately for exposed and
 9 unexposed groups if applicable. Report outcomes (phenotypes) for each
 10 genotype category
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 17 #16a Give unadjusted estimates and, if applicable, confounder-adjusted 10-11
 18 estimates and their precision (eg, 95% confidence interval). Make clear
 19 which confounders were adjusted for and why they were included
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 22 #16b Report category boundaries when continuous variables were categorized 10-11
 23
 24 #16c If relevant, consider translating estimates of relative risk into absolute 10-11
 25 risk for a meaningful time period
 26
 27
 28 #16d Report results of any adjustments for multiple comparisons 10-11
 29
 30 #17a Report other analyses done—e.g., analyses of subgroups and 10-11
 31 interactions, and sensitivity analyses
 32
 33 #17b Report other analyses done—e.g., analyses of subgroups and 10-11
 34 interactions, and sensitivity analyses
 35
 36 #17c Report other analyses done—e.g., analyses of subgroups and 10-11
 37 interactions, and sensitivity analyses
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 40 #18 Summarise key results with reference to study objectives 11-13
 41
 42
 43 #19 Discuss limitations of the study, taking into account sources of potential 13-14
 44 bias or imprecision. Discuss both direction and magnitude of any
 45 potential bias.
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 48 #20 Give a cautious overall interpretation considering objectives, limitations, 14
 49 multiplicity of analyses, results from similar studies, and other relevant
 50 evidence.
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 53 #21 Discuss the generalisability (external validity) of the study results 14
 54
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 56 #22 Give the source of funding and the role of the funders for the present 15
 57
 58
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1 study and, if applicable, for the original study on which the present article
2 is based
3

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6 tool made by the [EQUATOR Network](#) in collaboration with [Penelope.ai](#)
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BMJ Open

The association between genetically determined leptin and blood lipids considering alcohol consumption: a Mendelian randomization study

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Secondary Subject Heading:	Public health, Genetics and genomics
Keywords:	EPIDEMIOLOGY, leptin, lipids, alcohol consumption, genetic risk score

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5 2 **consumption: a Mendelian randomization study**
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8 3 **Running title: Genetically determined leptin and lipids**
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7
8 **Word count: 3,356**

Abstract

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

Design: We conducted a Mendelian randomization 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: 3,860 participants of the Framingham Heart Study 3rd 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) ($\beta=-0.66$, $p=0.01$), 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% confidence interval: 0.05-1.00) units decrease in log-TG. Besides significant association with log-TG ($\beta=-0.59$, $p=0.009$), the BMI dependent GRS was nominally associated with HDL-C ($\beta=-10.67$, $p=0.09$) and TC ($\beta=-28.05$, $p=0.08$). 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 ($p=0.03$), TG ($p=0.03$), and TC ($p=0.02$).

Conclusion: These findings together indicated that genetically determined leptin reduced lipid levels and the association may be modified by alcohol consumption.

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3 **1 Keywords:** leptin, lipids, alcohol consumption, genetic risk score
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6 **2 Strengths and limitations of this study:**
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9 • Population-based Mendelian randomization studies may offer an opportunity to provide
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11 better evidence for the association of leptin with lipid metabolism in the adult population
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13 compared with observational epidemiology studies.
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16 • The stringent quality control methods were used in measuring genotypes, phenotype, and
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18 covariates in the current study to reduce measurement error and increase the statistical
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20 power.
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23 • Pleiotropy effects of SNPs included in the leptin genetic risk score (GRS) may confound
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25 the leptin GRS and lipids associations.
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28 • Our analyses were restricted to individuals of European ancestry.
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Introduction

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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 extremely active endocrine organ, the adipose tissue secretes leptin playing a key role in immunometabolism.¹⁰ Leptin can regulate both innate and adaptive immune responses.^{11 12} Meanwhile, leptin and insulin interact to establish a regulatory feedback loop, the adipoinsular axis.¹³ Leptin suppresses insulin synthesis and secretion from β -cells¹³ and improves insulin sensitivity¹⁴. In turn, insulin can stimulate leptin secretion from adipocytes^{15 16}. Both the immune responses and insulin are involved in lipid metabolism.^{17 18} Case reports and case series have documented that leptin therapy can improve lipid profiles among patients with lipoatrophy or congenital leptin deficiency.¹⁹⁻²³ 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 triglycerides (TG).²⁴ 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.²⁵ A small clinical trial that involved 17 patients with HIV-associated lipodystrophy suggested that leptin treatment did not improve fasting lipid kinetics.²⁵ Population-based Mendelian randomization 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,²⁶ which provides an opportunity to conduct a Mendelian randomization study to delineate

1 the association between serum leptin and lipids levels. In addition, alcohol consumption has been
2 shown to influence leptin secretion in both human and animal models.²⁷⁻⁴⁰ In rodent models,
3 leptin has been demonstrated to be increased³⁰⁻³² or decreased^{33 34} after alcohol intake.
4 Similarly, leptin levels in human was decreased,³⁶ increased,^{35 37} or even unchanged³⁸⁻⁴⁰ after
5 drinking. It is unclear whether alcohol consumption modifies the association of genetically
6 determined leptin with lipid levels.^{41 42}

7 Therefore, the objectives of the current study were to evaluate the relationship between
8 genetically determined leptin and lipid levels and to explore whether the leptin-lipids
9 associations could be modified by alcohol consumption among participants of the Framingham
10 Heart Study (FHS) 3rd generation cohort.

11 **Materials and Methods**

12 **Data Sources and Study Participants**

13 The FHS was designed to identify common factors or characteristics that contribute to
14 cardiovascular disease (CVD) by tracking the development of CVD over a long period of time.
15 Participants of the FHS were free from overt symptoms of CVD or stroke at baseline. Later on,
16 the FHS was extended to including offspring and third generation of the original participants. A
17 detailed description of the FHS 3rd generation cohort has been outlined in previous
18 publications.⁴³ Genotype and phenotype data of the FHS are cataloged on the database of
19 genotype and phenotype (dbGaP) at the National Center for Biotechnology Information (NCBI).
20 We have received approval to use the FHS data by the Institutional Review Boards at the
21 University of Georgia and the NCBI. Circulating leptin levels, genotypes, lipid levels, and

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3 1 important covariates were available for 3,860 (94.7%) participants of the 3rd generation cohort at
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5 2 baseline in 2002-2005 (**Table 1**). Those participants were included in the current analyses.
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3 **Genotyping and Genetic Risk Score**

4 Genetic loci for circulating leptin levels have been reported in a large genome-wide
5 association studies (GWAS) meta-analysis by Kilpelainen and colleagues.²⁶ This study included
6 32,161 individuals of European ancestry and identified three single-nucleotide polymorphisms
7 (SNPs), *GCKR* rs780093, *LEP* rs10487505, and *SLC32A1* rs6071166, that were robustly
8 associated with body mass index (BMI) adjusted leptin at a genome-wide significance level
9 ($p < 5 \times 10^{-08}$). In addition, *GCKR* rs780093, *CCNLI* rs900400, and *FTO* rs8043757 were
10 associated with circulating leptin without adjustment for BMI.²⁶ We assumed the additive
11 genetic model for each SNP and constructed two genetic risk scores (GRSs) for leptin by
12 combining leptin-increasing alleles for SNPs weighted by their corresponding effect sizes on
13 logarithmically transformed leptin (log-leptin) as reported in the original GWAS meta-analysis.²⁶
14 The first score, GRS1, was generated using the three SNPs associated with BMI adjusted leptin,
15 and the second score, GRS2, using the three SNPs associated with leptin unadjusted for BMI.

16 Genome-wide SNPs were genotyped using Affymetrix and Illumina platforms in the
17 FHS. The 1000 Genome genotype data for the FHS was already imputed and cataloged on the
18 dbGaP. According to the document of the FHS,⁴⁴ before imputation, quality control removed
19 SNPs with a Hardy-Weinberg equilibrium $p < 1 \times 10^{-6}$, a missing rate $> 3.1\%$, a minor allele
20 frequency (MAF) $< 1\%$, a missing physical position or cannot mapped to build 37 positions,
21 Mendelian errors > 1000 , or duplicate SNPs. MACH software was used for genotype phasing,
22 followed by imputation using MiniMac software.^{45 46} Imputation results were summarized as

1 dosage scores, which represent the expected numbers of copies of the coded allele for each SNP,
2 ranging from 0 to 2. After imputation, SNPs with $r^2 < 0.30$, an MAF $< 1\%$, or a Hardy-Weinberg
3 equilibrium $p < 1 \times 10^{-6}$ were removed. We retrieved genotypes of the SNPs for GRSs from the
4 imputed data for all study participants (**Supplemental Table S1 and Supplemental Table S2**).

5 **Leptin and Lipids measurement**

6 In the FHS, blood samples were collected after overnight fasting and analyzed following
7 standard protocols.⁴⁷ Serum leptin levels were determined by enzyme-linked immunosorbent
8 assay (ELISA) method at R&D Systems using the Quantikine Human Leptin Immunoassay.⁴⁷
9 Leptin was logarithmically transformed for analyses in the current study.

10 Fasting blood lipids, including TC, HDL-C, and TG, were measured using automated
11 enzymatic assays.⁴⁷ For participants taking lipid-lowering medications, TC was adjusted as
12 TC/0.8.⁴⁸ After adjustment, LDL-C was calculated using the Friedewald formula.⁴⁹ The adjusted
13 TC and LDL-C and logarithmically transformed TG (log-TG) were used for analyses in the
14 current study.

15 **Covariates**

16 Demographic and health behavioral variables, including age, gender, education, smoking,
17 and drinking, were based on self-report. Education levels were categorized into “no more than
18 high school,” “some college,” and “bachelor’s degree or above.” Smoking was categorized into
19 “current smoker” or “not a current smoker” and drinking status into “current drinker” and “not a
20 current drinker.” Physical activity was measured with the physical activity index composite
21 score, which was calculated by summing the number of hours spent in each activity intensity

1 level weighted by their corresponding weight factor derived from the estimated oxygen
2 consumption requirement for each intensity level.⁵⁰ BMI was calculated as weight in kilograms
3 divided by the square of height in meters. Waist circumference was measured to next lower 1/4
4 inch by regional anthropometry.

5 **Statistical Analysis**

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

14 Three multivariate linear regression models were used to assess associations between log-
15 leptin and lipids, leptin GRS and log-leptin, and the leptin GRS and lipids, respectively. All
16 models were adjusted for age, sex, BMI, and waist circumference. To test robustness of the
17 leptin GRS and lipids associations, we additionally controlled for education, smoking, drinking,
18 and physical activity index score in the fully adjusted models. To explore whether associations
19 between the leptin GRS and lipids levels were modified by alcohol consumption, we performed
20 stratified analyses by drinking status. In each stratum of the drinking status, we tested
21 associations between leptin GRS and lipids by adjusting for age, sex, BMI, and waist
22 circumference in the base model and additionally adjusting for education, smoking, and physical

1 activity in the full model. Interactions between the leptin GRS and alcohol consumption were
2 tested among the overall participants by adding drinking and the interaction term, GRS×drinking,
3 to the models. All the above analyses were done for GRS1 and GRS2 separately. We quantified
4 the strength of the causal association of leptin with lipids using the instrumental variable
5 estimator.⁵¹ The estimator was calculated as the ratio of the coefficient for leptin GRS and lipids
6 association to the coefficient for the leptin GRS and log-leptin association from the base models.

7 To rule out the effect of lipid-lowering medications, sensitivity analyses were performed
8 among those not taking lipid medication. To rule out the effect of both diabetes and lipid-
9 lowering medications, sensitivity analyses were performed among those not taking lipid- or
10 glucose-lowering medications. All analyses were performed using SAS software (version 9.4;
11 SAS Institute Inc., Cary, North Carolina). Two-sided *p* values were provided, and *p*<0.05 was
12 considered significant.

13 **Participant and Public Involvement**

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

16 **Results**

17 Characteristics of the study participants are presented in **Table 1**. Participants were on
18 average 40.2 years old at baseline. There were slightly more females (53.2%), and only 15.4%
19 had less than a high school education. The majority (89.1%) of the participants were current
20 drinkers, and 15.6% were current smokers. Participants were on average over weighted, with a
21 mean BMI of 26.9 kg/m² and mean waist girth of 36.6 inches. About 6.9% of the participants

1 were treated for dyslipidemia, and 1.9% were treated for diabetes. The BMI independent leptin
2 GRS1 was not associated with age ($p=0.23$), sex ($p=0.89$), education ($p=0.22$), smoking
3 ($p=0.53$), drinking ($p=0.32$), BMI ($p=0.94$), waist circumference ($p=0.70$), lipid-lowering
4 medication usage ($p=0.26$), or the physical activity index score ($p=0.51$), but with diabetes-
5 lowering medication usage ($p=0.03$). As expected, the GRS1 was positively associated with age,
6 sex, BMI, and waist circumference adjusted log-leptin ($p=4.56\times 10^{-5}$).

7 **BMI independent leptin GRS1 and blood lipids**

8 After controlling for age, sex, BMI, and waist circumference, log-leptin was positively
9 associated with TC ($\beta=8.56$, $p=6.35\times 10^{-18}$), LDL-C ($\beta=6.46$, $p=1.85\times 10^{-13}$), and log-TG ($\beta=0.13$,
10 $p=1.59\times 10^{-20}$), but was not associated with HDL-C ($\beta=-0.62$, $p=0.11$) (**Figure 1 and**
11 **Supplemental Figure S1**). Per unit increase in the leptin GRS1 was associated with a 1.21-unit
12 increase in the age, sex, BMI, and waist circumference adjusted log-leptin ($p=4.56\times 10^{-5}$). The
13 leptin GRS1 was inversely associated with age, sex, BMI, and waist circumference adjusted log-
14 TG ($\beta=-0.66$, $p=0.01$) (**Figure 1**). When further adjusting for education, smoking, drinking, and
15 physical activity, the GRS1 and log-TG association was still significant ($\beta=-0.69$, $p=0.008$,
16 **Table 2**). Instrumental variable estimation indicated that log-TG levels decreased by 0.55 (95%
17 CI: 0.05, 1.00, $p=0.02$) per unit increase of genetically determined log-leptin level (**Figure 1**).
18 The leptin GRS1 was inversely associated with TC ($\beta=-12.50$, $p=0.49$) and LDL-C ($\beta=-0.11$,
19 $p=0.99$) and positively associated with HDL-C ($\beta=5.42$, $p=0.44$), however, the correlations were
20 not significant. The GRS1 and blood lipids associations were not modified by drinking status
21 (**Table 2**).

22 **BMI dependent leptin GRS2 and blood lipids**

1 As expected, the BMI dependent leptin GRS2 was not associated with any covariate
2 except for the BMI ($p=0.02$) and waist circumference ($p=0.03$). In the analyses controlling for
3 age, sex, BMI, and waist circumference, the GRS2 was significantly associated with lower levels
4 of log-TG ($p=0.009$) and nominally associated with lower levels of HDL-C ($p=0.09$) and TC
5 ($p=0.08$) (**Supplemental Figure S2**). When stratified by drinking status, the leptin GRS2 was
6 negatively associated with LDL-C ($\beta=-92.51$, $p=0.03$), log-TG ($\beta=-2.07$, $p=0.004$), and TC ($\beta=-$
7 144.68 , $p=0.003$) only among non-current drinkers (**Table 3**). When further adjusting for
8 education, smoking, drinking, and physical activity, those associations persisted (**Table 3**).
9 Furthermore, significant interactions between leptin GRS2 and alcohol drinking were identified
10 for LDL-C ($p=0.03$), log-TG ($p=0.03$), and TC ($p=0.02$) (**Table 3**).

11 When restricting to participants not taking lipid-lowering medication and those not taking
12 lipid- or glucose-lowering medications, respectively, the associations of GRS1 and GRS2 with
13 blood lipids were similar to those as shown above (**Supplemental Table S3, S4, S5 and S6**).

14 Discussion

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

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3 1 Both the BMI dependent- and independent- GRSs were associated with lower level of
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5 2 log-TG in the current study. Inconsistent associations between leptin and blood lipids have been
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7 3 observed in previous studies. In a small study of 80 postmenopausal women, serum leptin was
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9 4 positively associated with HDL-C, TG, and TC, and inversely associated with LDL-C.⁵² Another
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11 5 study conducted with 294 healthy school children reported that leptin was only associated with
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13 6 increased TG.⁵³ However, a study of 476 residents from Cameroon reported a positive
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15 7 correlation between leptin, LDL-C, and TC, and a positive association between leptin and TC,
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17 8 but no association between leptin and HDL-C or TG.⁵⁴ In a more recent study of 134 physically
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19 9 active postmenopausal women, no significant correlation was detected for leptin and blood
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21 10 lipids.⁵⁵ The divergent results of previous studies make it impossible to infer a relationship
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23 11 between leptin and blood lipids. Possible reasons for the divergent findings include varying
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25 12 sample sizes, failure to account for residual and unmeasured confounding, and the genetic
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27 13 background of the study population. Through Mendelian randomization analyses, we
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29 14 demonstrated that genetically determined leptin was inversely associated with log-TG. It is well
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31 15 known that alleles, such as risk alleles for leptin, are randomly assigned at meiosis and therefore,
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33 16 are independent of non-genetic confounders. The association between leptin GRS and log-TG in
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35 17 the current study was less prone to confounding. This also highlights the importance of using
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37 18 Mendelian randomization to delineate causal relationships. Our finding is further supported by
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39 19 previous physiologic studies, among which, leptin was demonstrated to inhibit lipogenesis,
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41 20 stimulate lipolysis, and reduce triglyceride uptake.⁵⁶ However, the association of HDL-C and TC
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43 21 were only nominally significant with BMI dependent GRS2 in the current study. It could be due
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45 22 to lack of statistical power or existing interaction of leptin and drinking. Therefore, we cannot
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47 23 rule out causal relationships between leptin and those lipid measures. Future large-scale
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3 1 Mendelian randomization studies are warranted to evaluate associations of leptin GRS with
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9 3 The BMI independent GRS1 was only associated with log-TG, while the BMI dependent
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11 4 GRS2 was also in nominal associations with HDL-C and TC. In addition, alcohol drinking
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13 5 modified the GRS2-lipids associations but not the GRS1-lipids associations. This indicated that
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15 6 the role of leptin in blood lipids regulation may be through multiple mechanisms. The BMI
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17 7 dependent GRS2 were inversely associated with LDL-C, log-TG, and TC only among non-
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19 8 current drinkers, but not among current drinkers. Although future studies are warranted to
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21 9 confirm these interactions, previous physiological studies may provide a reasonable explanation.
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23 10 Singh and colleagues demonstrated that the increased expression of caveolin-1 impairs leptin
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25 11 signaling and attenuates leptin-dependent effect to prevent lipid accumulation in human white
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27 12 pre-adipocytes.⁵⁷ Meanwhile, Caveolin-1 can be increased by alcohol drinking.⁵⁸
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33 13 Our study represents the first Mendelian randomization analyses for leptin and blood
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35 14 lipids in a population of European ancestry. A major strength of this study is the stringent quality
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37 15 control methods used in measuring genotypes, phenotype, and covariates in the FHS 3rd
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39 16 Generation Cohort. Those methods can reduce measurement error and increase the statistical
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41 17 power needed to identify associations between leptin GRS and lipids. We also identify some
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43 18 limitations. First, pleiotropy effects of SNPs included in the leptin GRS may confound the leptin
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45 19 GRS and lipids associations. It is possible that our results may represent a shared genetic basis
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47 20 between leptin and lipids rather than a causal relationship. Second, we may not have sufficient
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49 21 power to detect associations between genetically determined leptin levels and LDL-C, HDL-C,
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51 22 and TC. Larger Mendelian randomization studies are warranted to evaluate associations between
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1 leptin and LDL-C, HDL-C, and TC. Third, we did not control for total energy intake in our
2 analyses because food frequency questionnaire survey was not conducted in the third generation
3 cohort at baseline when leptin was measured. However, leptin combines with receptors in the
4 hypothalamus to reduce appetite and increase energy expenditure. Therefore, total energy intake
5 is in the pathway from leptin to lipids metabolism and may not meet the criteria of being a
6 confounder. Forth, the type of alcohol consumed was not measured and cannot be considered in
7 the current analyses. It is possible that the alcohol consumed in the studied population is mainly
8 wine and/or beers, which contain high level of resveratrol and phytochemical. The two chemicals
9 may benefit lipid metabolism.^{59 60} However, the two chemicals do not share similar genetic
10 profile with leptin, and consequently, they should not be correlated with leptin and cannot affect
11 the associations between leptin GRS and blood lipids. Finally, our analyses were restricted to
12 individuals of European ancestry. Our findings may not be generalizable to populations of other
13 ancestries.

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

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20 **Author Contributions**

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3 1 Conceptualization, Changwei Li, José Cordero, Jia-Sheng Wang, Shengxu Li, and Zhi-
4 Yong Zou; Formal analysis, Luqi Shen and Ye Shen; Supervision, Changwei Li, José Cordero,
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6 2 Jia-Sheng Wang, Shengxu Li, and Zhi-Yong-Zou; Writing – original draft, Luqi Shen; Writing –
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8 3 review & editing, Changwei Li, José Cordero, Luqi Shen, Lirong Liang, and Zhi-Yong Zou.
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13 5 **Conflict of interest**

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17 6 Conflicts of interest and disclosures: none.
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26 9 and China Scholarship Council (201806015008).
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30 10 **Data sharing statement**

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33 11 The deidentified dataset supporting this study is available on the database of genotype
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35 12 and phenotype (dbGaP) at the National Center for Biotechnology Information (NCBI).
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38 13 https://www.ncbi.nlm.nih.gov/projects/gap/cgi-bin/study.cgi?study_id=phs000007.v30.p11. The
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40 14 researchers are able to reuse the dataset on the condition that they get the approval from dbGaP
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Figure legends

Figure 1. The relationship between leptin, genetic risk score for leptin and triglycerides (TG) in Framingham Heart Study the 3rd Generation cohort.

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Table 1. Characteristics of the Study Participants by Genetic Risk Score 1 (GRS1)^a for logarithmically transformed Leptin in Framingham Heart Study 3rd Generation Cohort.

Covariates	Overall	Quartiles of the leptin GRS				P
	(n=3,860)	Q1 (n=964)	Q2 (n=961)	Q3 (n=977)	Q4 (n=958)	
Genetic risk score, 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 (%)						
<i>No more than high school</i>	591 (15.4)	141 (14.7)	146 (15.3)	157 (16.1)	147 (15.4)	
<i>Some college</i>	1213 (31.5)	306 (31.8)	287 (30.0)	313 (32.1)	307 (32.3)	0.22
<i>Bachelor's degree and above</i>	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
Low Density Lipoprotein, 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
High Density Lipoprotein, 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
Triglycerides, 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*
Total Cholesterol, 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)	2.0 (1.1)	2.0 (1.0)	2.0 (1.1)	2.0 (1.0)	2.1 (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

BMI=body mass index; Log-leptin=logarithmically transformed leptin; GRS=Genetic Risk Score; SD=standard deviation.

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3^a Genetic risk scores 1(GRS1) for leptin was generated by summing leptin increasing alleles of 3 SNPs adjusted for BMI, weighted by
4 their corresponding effect sizes reported by Kilpelainen et al.

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6 *Log transformed leptin and triglycerides were used to calculate the *P*-values.
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Table 2. Association of BMI independent Leptin GRS1^a with Baseline Lipids among Overall, Drinking, and Non-Drinking Participants of the Framingham Heart Study 3rd Generation Cohort, Respectively.

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

GRS=Genetic Risk Score; HDL-C =High-density lipoprotein cholesterol; LDL-C =Low-density lipoprotein cholesterol; log-TG=logarithmically transformed triglycerides; SE=standard error.

^a Genetic risk scores1 (GRS1) for leptin was generated by summing leptin increasing alleles of 3 SNPs adjusted for body mass index (BMI), weighted by their corresponding effect sizes reported by Kilpelainen et al;

^b 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;

^c Adjusted for age, sex, education, smoking, drinking, BMI, waist, and physical activity among the overall sample;

^d Assessed by adding an interaction term, drinking×GRS to the fully adjusted model among the overall participants.

Table 3. Association of BMI dependent Leptin GRS^{2a} with Baseline Lipids among Overall, Drinking, and Non-Drinking Participants of the Framingham Heart Study 3rd Generation Cohort, Respectively.

	Age, sex, BMI, waist adjusted model		$P_{\text{interaction}}^b$	Fully adjusted model ^c		$P_{\text{interaction}}^d$
	Beta(SE)	P		Beta(SE)	P	
HDL-C						
Overall	-10.67 (6.20)	0.09		-10.98 (6.22)	0.08	
Not current drinkers	-0.69 (16.31)	0.97	0.56	0.82 (16.55)	0.96	0.52
Current drinkers	-12.15 (6.64)	0.07		-11.94 (6.68)	0.07	
LDL-C						
Overall	-2.11 (14.05)	0.88		-2.81 (14.21)	0.84	
Not current drinkers	-92.51 (43.02)	0.03	0.03	-101.15 (43.78)	0.02	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		-0.59 (0.23)	0.01	
Not current drinkers	-2.07 (0.71)	0.004	0.03	-2.03 (0.72)	0.005	0.03
Current drinkers	-0.40 (0.24)	0.09		-0.42 (0.24)	0.08	
Total cholesterol						
Overall	-28.05 (15.91)	0.08		-28.74 (16.02)	0.07	
Not current drinkers	-144.68 (47.61)	0.003	0.02	-151.32 (48.37)	0.002	0.01
Current drinkers	-13.19 (16.90)	0.44		-14.67 (16.98)	0.39	

GRS=Genetic Risk Score; HDL-C =High-density lipoprotein cholesterol; LDL-C =Low-density lipoprotein cholesterol; log-TG=logarithmically transformed triglycerides; SE=standard error.

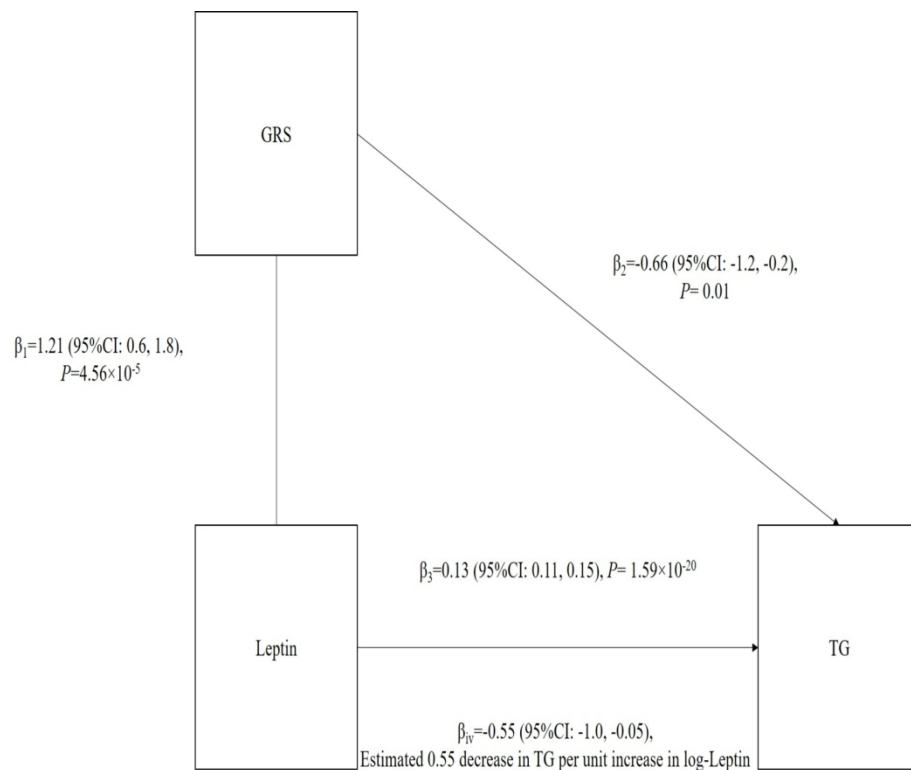
^a Genetic risk scores² (GRS²) for leptin was generated by summing leptin increasing alleles of 3 SNPs unadjusted for body mass index (BMI), weighted by their corresponding effect sizes reported by Kilpelainen et al;

^b 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;

^c Adjusted for age, sex, education, smoking, drinking, BMI, waist, and physical activity among the overall sample;

^d Assessed by adding an interaction term, drinking×GRS to the fully adjusted model among the overall participants.

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Supplemental Table S1. Basic Information of three SNPs for BMI independent leptin GRS1^a reaching genome-wide significance ($P < 5 \times 10^{-8}$)

Chromosome		Coded	Non-coded	Nearest			
Position	rsID	Allele	Allele	R ²	Function	Gene	Population
2:27742603	rs780093	C	T	0.995	intron variant	GCKR	European American
7:127860163	rs10487505	G	C	0.989	intron variant	LEP	European American
20:37333012	rs6071166	C	A	0.973	intergenic	SLC32A1	European American

BMI=body mass index

^a Genetic risk scores 1 (GRS1) for leptin was generated by summing leptin increasing alleles of 3 SNPs adjusted for body mass index (BMI), weighted by their corresponding effect sizes reported by Kilpelainen et al.

Supplemental Table S2. Basic Information of three SNPs for BMI dependent leptin GRS2^a reaching genome-wide significance (P<5×10⁻⁸)

Chromosome		Coded	Non-coded			Nearest	
Position	rsID	Allele	Allele	R²	Function	Gene	Population
2:27742603	rs780093	C	T	0.995	intron variant	GCKR	European American
3:156798775	rs900400	T	C	0.691	upstream variant 2KB	CCNL1	European American
16:53813450	rs8043757	A	T	0.999	intron variant	FTO	European American

BMI=body mass index

^a Genetic risk scores 2 (GRS2) for leptin was generated by summing leptin increasing alleles of 3 SNPs unadjusted for body mass index (BMI), weighted by their corresponding effect sizes reported by Kilpelainen et al.

Supplemental Table S3. Association of BMI independent Leptin GRS1^a with Baseline Lipids among Overall, Drinking, and Non-Drinking Participants without treating for Lipids of the Framingham Heart Study 3rd Generation Cohort, Respectively.

	Age, sex, BMI, waist adjusted model		<i>P</i> _{interaction^b}	Fully adjusted model ^c		<i>P</i> _{interaction^d}
	Beta(SE)	<i>P</i>		Beta(SE)	<i>P</i>	
HDL-C						
Overall	5.25 (7.44)	0.48		7.59 (7.47)	0.31	
Not current drinkers	18.83 (19.59)	0.34	0.86	20.43 (20.23)	0.31	0.83
Current drinkers	6.22 (7.97)	0.44		7.19 (8)	0.37	
LDL-C						
Overall	14.86 (16.06)	0.35		13.43 (16.2)	0.41	
Not current drinkers	36.1 (49.22)	0.46	0.51	27.18 (50.69)	0.59	0.61
Current drinkers	12.19 (17.05)	0.47		10.48 (17.11)	0.54	
log-TG						
Overall	-0.69 (0.27)	0.01		-0.72 (0.27)	0.007	
Not current drinkers	-1.72 (0.84)	0.04	0.20	-1.61 (0.86)	0.06	0.21
Current drinkers	-0.58 (0.28)	0.04		-0.6 (0.28)	0.03	

Total cholesterol

Overall	-0.09 (18.19)	1.00		-0.67 (18.26)	0.97	
Not current drinkers	3.78 (55.05)	0.95	0.94	-2.29 (56.42)	0.97	0.97
Current drinkers	0.98 (19.29)	0.96		-0.34 (19.32)	0.99	

BMI=body mass index; GRS=Genetic Risk Score; HDL-C=High-density lipoprotein cholesterol; LDL-C=Low-density lipoprotein cholesterol; log-TG=logarithmically transformed triglycerides; SE=standard error

^a Genetic risk scores 1 (GRS1) for leptin was generated by summing leptin increasing alleles of 3 SNPs adjusted for body mass index (BMI), weighted by their corresponding effect sizes reported by Kilpelainen et al;

^b 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;

^c Adjusted for age, sex, education, smoking, drinking, BMI, waist, and physical activity among the overall sample;

^d Assessed by adding an interaction term, drinking×GRS to the fully adjusted model among the overall participants.

Supplemental Table S4. Association of BMI dependent Leptin GRS2^a with Baseline Lipids among Overall, Drinking, and Non-Drinking Participants without treating for Lipids of the Framingham Heart Study 3rd Generation Cohort, Respectively.

	Age, sex, BMI, waist adjusted model			Fully adjusted model ^c		
	Beta(SE)	<i>P</i>	<i>P</i> _{interaction} ^b	Beta(SE)	<i>P</i>	<i>P</i> _{interaction} ^d
HDL-C						
Overall	-9.73 (6.50)	0.13		-9.66 (6.53)	0.14	
Not current drinkers	-2.61 (17.44)	0.88	0.72	-1.22 (17.77)	0.95	0.68
Current drinkers	-10.52 (6.94)	0.13		-10.19 (6.99)	0.15	
LDL-C						
Overall	-0.42 (14.02)	0.98		-1.56 (14.17)	0.91	
Not current drinkers	-83.19 (43.58)	0.06	0.07	-94.12 (44.22)	0.03	0.05
Current drinkers	9.71 (14.86)	0.51		7.61 (14.96)	0.61	
log-TG						
Overall	-0.63 (0.23)	0.007		-0.63 (0.23)	0.007	
Not current drinkers	-2.09 (0.74)	0.005	0.04	-2.00 (0.75)	0.008	0.05
Current drinkers	-0.45 (0.25)	0.07		-0.47 (0.25)	0.05	
Total cholesterol						

Overall	-25.28 (15.87)	0.11		-26.38 (15.97)	0.10	
Not current drinkers	-136.15 (48.44)	0.005	0.03	-144.29 (48.92)	0.003	0.02
Current drinkers	-10.96 (16.82)	0.51		-13.64 (16.90)	0.42	

BMI=body mass index; GRS=Genetic Risk Score; HDL-C=High-density lipoprotein cholesterol; LDL-C=Low-density lipoprotein cholesterol; log-TG=logarithmically transformed triglycerides; SE=standard error

^a Genetic risk scores 2 (GRS2) for leptin was generated by summing leptin increasing alleles of 3 SNPs unadjusted for body mass index (BMI), weighted by their corresponding effect sizes reported by Kilpelainen et al;

^b 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;

^c Adjusted for age, sex, education, smoking, drinking, BMI, waist, and physical activity among the overall sample;

^d Assessed by adding an interaction term, drinking×GRS to the fully adjusted model among the overall participants.

Supplemental Table S5. Association of BMI independent Leptin GRS1^a with Baseline Lipids among Overall, Drinking, and Non-Drinking Participants without treating for Lipids and Diabetes of the Framingham Heart Study 3rd Generation Cohort, Respectively.

	Age, sex, BMI, waist adjusted model			Fully adjusted model ^c		
	Beta(SE)	<i>P</i>	<i>P</i> _{interaction} ^b	Beta(SE)	<i>P</i>	<i>P</i> _{interaction} ^d
HDL-C						
Overall	5.64 (7.47)	0.45		8.00 (7.49)	0.29	
Not current drinkers	20.89 (19.62)	0.29	0.72	22.51 (20.25)	0.27	0.69
Current drinkers	6.22 (7.98)	0.44		7.17 (8.02)	0.37	
LDL-C						
Overall	15.29 (16.11)	0.34		13.42 (16.27)	0.41	
Not current drinkers	33.51 (49.77)	0.50	0.58	24.63 (51.26)	0.63	0.68
Current drinkers	12.68 (17.09)	0.46		10.99 (17.16)	0.52	
log-TG						
Overall	-0.66 (0.27)	0.01		-0.69 (0.27)	0.01	
Not current drinkers	-1.60 (0.85)	0.06	0.23	-1.46 (0.86)	0.09	0.25
Current drinkers	-0.57 (0.28)	0.04		-0.59 (0.28)	0.04	

Total cholesterol

Overall	1.17 (18.24)	0.95		0.14 (18.33)	0.99	
Not current drinkers	6.19 (55.64)	0.91	0.93	1.04 (57.03)	0.99	0.99
Current drinkers	1.56 (19.35)	0.94		0.26 (19.38)	0.99	

BMI=body mass index; GRS=Genetic Risk Score; HDL-C =High-density lipoprotein cholesterol; LDL-C =Low-density lipoprotein cholesterol; log-TG=logarithmically transformed triglycerides; SE=standard error

^a Genetic risk scores 1 (GRS1) for leptin was generated by summing leptin increasing alleles of 3 SNPs adjusted for body mass index (BMI), weighted by their corresponding effect sizes reported by Kilpelainen et al;

^b 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;

^c Adjusted for age, sex, education, smoking, drinking, BMI, waist, and physical activity among the overall sample;

^d Assessed by adding an interaction term, drinking×GRS to the fully adjusted model among the overall participants.

Supplemental Table S6. Association of BMI dependent Leptin GRS2^a with Baseline Lipids among Overall, Drinking, and Non-Drinking Participants without treating for Lipids and Diabetes of the Framingham Heart Study 3rd Generation Cohort, respectively.

	Age, sex, BMI, waist adjusted model		<i>P</i> _{interaction^b}	Fully adjusted model ^c		<i>P</i> _{interaction^d}
	Beta(SE)	<i>P</i>		Beta(SE)	<i>P</i>	
HDL-C						
Overall	-10.39 (6.52)	0.11		-10.48 (6.55)	0.11	
Not current drinkers	-8.18 (17.54)	0.64	0.95	-7.77 (17.87)	0.66	0.92
Current drinkers	-10.58 (6.96)	0.13		-10.32 (7.01)	0.14	
LDL-C						
Overall	-1.58 (14.07)	0.91		-3.3 (14.22)	0.82	
Not current drinkers	-95.04 (44.19)	0.03	0.04	-105.66 (44.81)	0.02	0.03
Current drinkers	8.84 (14.90)	0.55		6.84 (15.00)	0.65	
log-TG						
Overall	-0.62 (0.23)	0.008		-0.62 (0.23)	0.008	
Not current drinkers	-2.00 (0.75)	0.008	0.06	-1.87 (0.76)	0.01	0.08

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3	Current drinkers	-0.45 (0.25)	0.07		-0.48 (0.25)	0.05	
4							
5	Total cholesterol						
6							
7	Overall	-26.72 (15.92)	0.09		-28.67 (16.03)	0.07	
8							
9	Not current drinkers	-150.52 (49.04)	0.002	0.01	-158.43 (49.51)	0.002	0.01
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11	Current drinkers	-12.06 (16.87)	0.47		-14.69 (16.95)	0.39	
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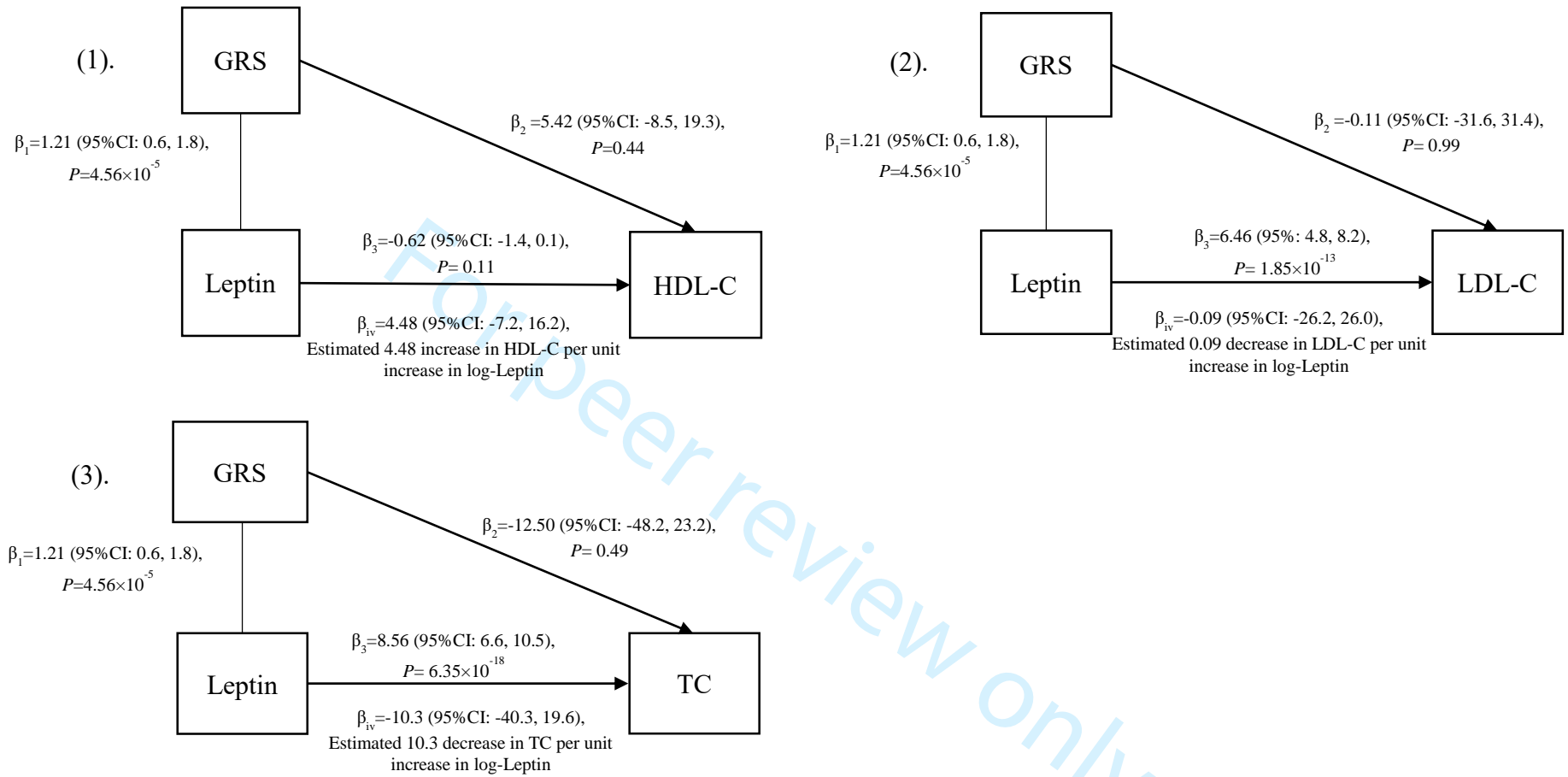
BMI=body mass index; GRS=Genetic Risk Score; HDL-C=High-density lipoprotein cholesterol; LDL-C=Low-density lipoprotein cholesterol; log-TG=logarithmically transformed triglycerides; SE=standard error

^a Genetic risk scores 2 (GRS2) for leptin was generated by summing leptin increasing alleles of 3 SNPs unadjusted for body mass index (BMI), weighted by their corresponding effect sizes reported by Kilpelainen et al;

^b 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;

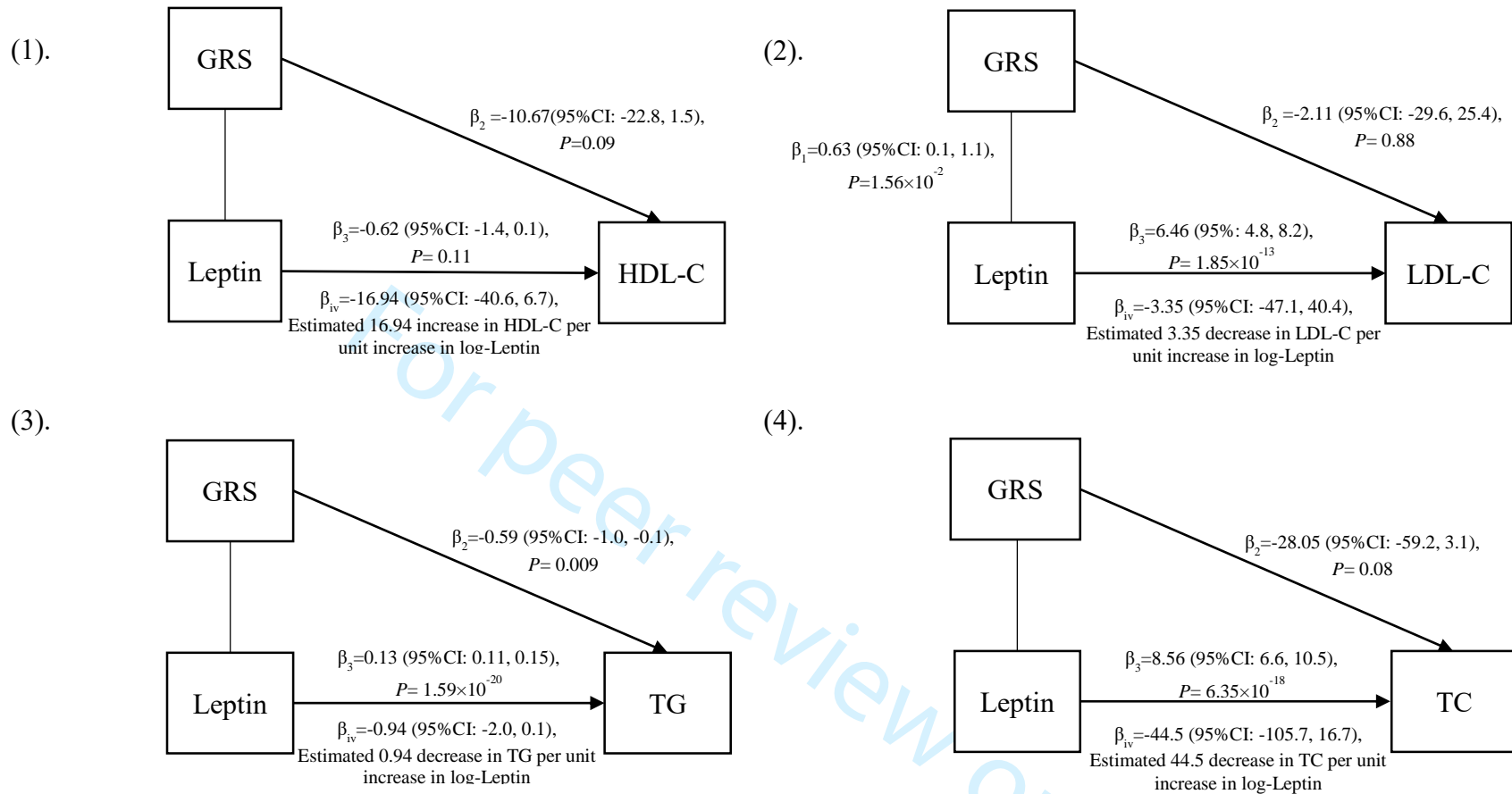
^c Adjusted for age, sex, education, smoking, drinking, BMI, waist, and physical activity among the overall sample;

^d Assessed by adding an interaction term, drinking×GRS to the fully adjusted model among the overall participants.



Supplemental Figure S1. The relationship between Leptin, Genetic Risk Score 1 (GRS1) for Leptin and Lipids in Framingham Heart Study the 3rd Generation cohort.

1. The relationship between leptin, genetic risk score for leptin and high density lipoprotein cholesterol (HDL-C)
2. The relationship between leptin, genetic risk score for leptin and low density lipoprotein cholesterol (LDL-C)
3. The relationship between leptin, genetic risk score for leptin and total cholesterol (TC)



Supplemental Figure S2. The relationship between Leptin, Genetic Risk Score 2 (GRS2) for Leptin and Lipids in Framingham Heart Study the 3rd Generation cohort.

1. The relationship between leptin, genetic risk score for leptin and high density lipoprotein cholesterol (HDL-C)
2. The relationship between leptin, genetic risk score for leptin and low density lipoprotein cholesterol (LDL-C)
3. The relationship between leptin, genetic risk score for leptin and triglycerides (TG)

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4. The relationship between leptin, genetic risk score for leptin and total cholesterol (TC)

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Reporting checklist for genetic association study.

Based on the STREGA guidelines.

Instructions to authors

Complete this checklist by entering the page numbers from your manuscript where readers will find each of the items listed below.

Your article may not currently address all the items on the checklist. Please modify your text to include the missing information. If you are certain that an item does not apply, please write "n/a" and provide a short explanation.

Upload your completed checklist as an extra file when you submit to a journal.

In your methods section, say that you used the STREGA reporting guidelines, and cite them as:

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		Reporting Item	Page Number
Title	#1a	Indicate the study's design with a commonly used term in the title or the abstract	1
Abstract	#1b	Provide in the abstract an informative and balanced summary of what was done and what was found	2
	#2	Explain the scientific background and rationale for the investigation being reported	4
	#3	State specific objectives, including any prespecified hypotheses. State if the study is the first report of a genetic association, a replication effort, or both.	5
	#4	Present key elements of study design early in the paper	5
	#5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	5-6

1	#6a	Cohort study – Give the eligibility criteria, and the sources and methods	5-6
2		of selection of participants. Describe methods of follow-up. Case-control	
3		study – Give the eligibility criteria, and the sources and methods of case	
4		ascertainment and control selection. Give the rationale for the choice of	
5		cases and controls. Cross-sectional study – Give the eligibility criteria,	
6		and the sources and methods of selection of participants. Give	
7		information on the criteria and methods for selection of subsets of	
8		participants from a larger study, when relevant.	
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14	#6b	Cohort study – For matched studies, give matching criteria and number	n/a
15		of exposed and unexposed. Case-control study – For matched studies,	
16		give matching criteria and the number of controls per case.	
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19	#7a	Clearly define all outcomes, exposures, predictors, potential	7-8
20		confounders, and effect modifiers. Give diagnostic criteria, if applicable	
21			
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23	#7b	Clearly define genetic exposures (genetic variants) using a widely-used	6-7
24		nomenclature system. Identify variables likely to be associated with	
25		population stratification (confounding by ethnic origin).	
26			
27			
28	#8a	For each variable of interest give sources of data and details of methods	5-6
29		of assessment (measurement). Describe comparability of assessment	
30		methods if there is more than one group. Give information separately for	
31		for exposed and unexposed groups if applicable.	
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35	#8b	Describe laboratory methods, including source and storage of DNA,	6-7
36		genotyping methods and platforms (including the allele calling algorithm	
37		used, and its version), error rates and call rates. State the laboratory /	
38		centre where genotyping was done. Describe comparability of laboratory	
39		methods if there is more than one group. Specify whether genotypes	
40		were assigned using all of the data from the study simultaneously or in	
41		smaller batches.	
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46	#9a	Describe any efforts to address potential sources of bias	8-9
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48	#9b	Describe any efforts to address potential sources of bias	8-9
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51	#10	Explain how the study size was arrived at	5-6
52			
53	#11	Explain how quantitative variables were handled in the analyses. If	6
54		applicable, describe which groupings were chosen, and why. If	
55		applicable, describe how effects of treatment were dealt with.	
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59	#12a	Describe all statistical methods, including those used to control for	8-9
60		For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml	

1	confounding. State software version used and options (or settings)	
2	chosen.	
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4	#12b Describe any methods used to examine subgroups and interactions	9
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6	#12c Explain how missing data were addressed	9
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8	#12d If applicable, explain how loss to follow-up was addressed	n/a
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11	#12e Describe any sensitivity analyses	9
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13	#12f State whether Hardy-Weinberg equilibrium was considered and, if so,	7
14	how.	
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17	#12g Describe any methods used for inferring genotypes or haplotypes	7
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19	#12h Describe any methods used to assess or address population	7
20	stratification.	
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23	#12i Describe any methods used to address multiple comparisons or to	7
24	control risk of false positive findings.	
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27	#12j Describe any methods used to address and correct for relatedness	n/a
28	among subjects	
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31	#13a Report numbers of individuals at each stage of study—eg numbers	5-6
32	potentially eligible, examined for eligibility, confirmed eligible, included in	
33	the study, completing follow-up, and analysed. Give information	
34	separately for for exposed and unexposed groups if applicable. Report	
35	numbers of individuals in whom genotyping was attempted and numbers	
36	of individuals in whom genotyping was successful.	
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41	#13b Give reasons for non-participation at each stage	n/a
42		
43	#13c Consider use of a flow diagram	n/a
44		
45	#14a Give characteristics of study participants (eg demographic, clinical,	9-10
46	social) and information on exposures and potential confounders. Give	
47	information separately for exposed and unexposed groups if applicable.	
48	Consider giving information by genotype	
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52	#14b Indicate number of participants with missing data for each variable of	9-10
53	interest	
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56	#14c Cohort study – Summarize follow-up time, e.g. average and total	n/a
57	amount.	
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- 1 #15 Cohort study Report numbers of outcome events or summary measures 9/10
 2 over time. Give information separately for exposed and unexposed
 3 groups if applicable. Report outcomes (phenotypes) for each genotype
 4 category over time Case-control study – Report numbers in each
 5 exposure category, or summary measures of exposure. Give information
 6 separately for cases and controls . Report numbers in each genotype
 7 category. Cross-sectional study – Report numbers of outcome events or
 8 summary measures. Give information separately for exposed and
 9 unexposed groups if applicable. Report outcomes (phenotypes) for each
 10 genotype category
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 17 #16a Give unadjusted estimates and, if applicable, confounder-adjusted 10-11
 18 estimates and their precision (eg, 95% confidence interval). Make clear
 19 which confounders were adjusted for and why they were included
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 22 #16b Report category boundaries when continuous variables were categorized 10-11
 23
 24 #16c If relevant, consider translating estimates of relative risk into absolute 10-11
 25 risk for a meaningful time period
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 28 #16d Report results of any adjustments for multiple comparisons 10-11
 29
 30 #17a Report other analyses done—e.g., analyses of subgroups and 10-11
 31 interactions, and sensitivity analyses
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 33 #17b Report other analyses done—e.g., analyses of subgroups and 10-11
 34 interactions, and sensitivity analyses
 35
 36 #17c Report other analyses done—e.g., analyses of subgroups and 10-11
 37 interactions, and sensitivity analyses
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 40 #18 Summarise key results with reference to study objectives 11-13
 41
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 43 #19 Discuss limitations of the study, taking into account sources of potential 13-14
 44 bias or imprecision. Discuss both direction and magnitude of any
 45 potential bias.
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 48 #20 Give a cautious overall interpretation considering objectives, limitations, 14
 49 multiplicity of analyses, results from similar studies, and other relevant
 50 evidence.
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 53 #21 Discuss the generalisability (external validity) of the study results 14
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 56 #22 Give the source of funding and the role of the funders for the present 15
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1 study and, if applicable, for the original study on which the present article
2 is based
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5 CC-BY. This checklist was completed on 22. September 2018 using <http://www.goodreports.org/>, a
6 tool made by the [EQUATOR Network](#) in collaboration with [Penelope.ai](#)
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BMJ Open

The association between genetically determined leptin and blood lipids considering alcohol consumption: a Mendelian randomization study

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Primary Subject Heading:	Epidemiology
Secondary Subject Heading:	Public health, Genetics and genomics
Keywords:	EPIDEMIOLOGY, leptin, lipids, alcohol consumption, genetic risk score

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3 1 **The association between genetically determined leptin and blood lipids considering alcohol**
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5 **consumption: a Mendelian randomization study**
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8 **Running title: Genetically determined leptin and lipids**
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7
8 **Word count: 3,356**

Abstract

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

Design: We conducted a Mendelian randomization 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: 3,860 participants of the Framingham Heart Study 3rd 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) ($\beta=-0.66$, $p=0.01$), 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% confidence interval: 0.05-1.00) units decrease in log-TG. Besides significant association with log-TG ($\beta=-0.59$, $p=0.009$), the BMI dependent GRS was nominally associated with HDL-C ($\beta=-10.67$, $p=0.09$) and TC ($\beta=-28.05$, $p=0.08$). 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 ($p=0.03$), 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.

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2
3 **1 Keywords:** leptin, lipids, alcohol consumption, genetic risk score
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6 **2 Strengths and limitations of this study:**
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- 8
9 • Population-based Mendelian randomization studies may offer an opportunity to provide
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11 better evidence for the association of leptin with lipid metabolism in the adult population
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13 compared with observational epidemiology studies.
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16 • The stringent quality control methods were used in measuring genotypes, phenotype, and
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18 covariates in the current study to reduce measurement error and increase the statistical
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20 power.
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23 • Pleiotropy effects of SNPs included in the leptin genetic risk score (GRS) may confound
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25 the leptin GRS and lipids associations.
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28 • Our analyses were restricted to individuals of European ancestry.
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Introduction

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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 hyperleptinemia decreases the expression of SREBP-1c, a master regulator of lipid metabolism, in liver and adenovirus-induced hyperleptinemia decreases triglyceride synthesis through SREBP-1c down-regulation.¹³ Meanwhile, SREBP-1c is involved in innate immune response in Macrophages¹⁴. 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 triglycerides (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 randomization 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

1 leptin,²² which provides an opportunity to conduct a Mendelian randomization study to delineate
2 the association between serum leptin and lipids levels. In addition, alcohol consumption has been
3 shown to influence leptin secretion in both human and animal models.²³⁻³⁶ In rodent models,
4 leptin has been demonstrated to be increased²⁶⁻²⁸ or decreased^{29 30} after alcohol intake.
5 Similarly, leptin levels in human was decreased,³² increased,^{31 33} or even unchanged³⁴⁻³⁶ after
6 drinking. It is unclear whether alcohol consumption modifies the association of genetically
7 determined leptin with lipid levels.^{37 38}

8 Therefore, the objectives of the current study were to evaluate the relationship between
9 genetically determined leptin and lipid levels and to explore whether the leptin-lipids
10 associations could be modified by alcohol consumption among participants of the Framingham
11 Heart Study (FHS) 3rd generation cohort.

12 **Materials and Methods**

13 **Data Sources and Study Participants**

14 The FHS was designed to identify common factors or characteristics that contribute to
15 cardiovascular disease (CVD) by tracking the development of CVD over a long period of time.
16 Participants of the FHS were free from overt symptoms of CVD or stroke at baseline. Later on,
17 the FHS was extended to including offspring and third generation of the original participants. A
18 detailed description of the FHS 3rd generation cohort has been outlined in previous
19 publications.³⁹ Genotype and phenotype data of the FHS are cataloged on the database of
20 genotype and phenotype (dbGaP) at the National Center for Biotechnology Information (NCBI).
21 We have received approval to use the FHS data by the Institutional Review Boards at the
22 University of Georgia and the NCBI. Circulating leptin levels, genotypes, lipid levels, and

1 important covariates were available for 3,860 (94.7%) participants of the 3rd generation cohort at
2 baseline in 2002-2005 (**Table 1**). Those participants were included in the current analyses.

3 **Genotyping and Genetic Risk Score**

4 Genetic loci for circulating leptin levels have been reported in a large genome-wide
5 association studies (GWAS) meta-analysis by Kilpelainen and colleagues.²² This study included
6 32,161 individuals of European ancestry and identified three single-nucleotide polymorphisms
7 (SNPs), *GCKR* rs780093, *LEP* rs10487505, and *SLC32A1* rs6071166, that were robustly
8 associated with body mass index (BMI) adjusted leptin at a genome-wide significance level
9 ($p < 5 \times 10^{-08}$). In addition, *GCKR* rs780093, *CCNLI* rs900400, and *FTO* rs8043757 were
10 associated with circulating leptin without adjustment for BMI.²² We assumed the additive
11 genetic model for each SNP and constructed two genetic risk scores (GRSs) for leptin by
12 combining leptin-increasing alleles for SNPs weighted by their corresponding effect sizes on
13 logarithmically transformed leptin (log-leptin) as reported in the original GWAS meta-analysis.²²
14 The first score, GRS1, was generated using the three SNPs associated with BMI adjusted leptin,
15 and the second score, GRS2, using the three SNPs associated with leptin unadjusted for BMI.

16 Genome-wide SNPs were genotyped using Affymetrix and Illumina platforms in the
17 FHS. The 1000 Genome genotype data for the FHS was already imputed and cataloged on the
18 dbGaP. According to the document of the FHS,⁴⁰ before imputation, quality control removed
19 SNPs with a Hardy-Weinberg equilibrium $p < 1 \times 10^{-6}$, a missing rate $> 3.1\%$, a minor allele
20 frequency (MAF) $< 1\%$, a missing physical position or cannot mapped to build 37 positions,
21 Mendelian errors > 1000 , or duplicate SNPs. MACH software was used for genotype phasing,
22 followed by imputation using MiniMac software.^{41 42} Imputation results were summarized as

1 dosage scores, which represent the expected numbers of copies of the coded allele for each SNP,
2 ranging from 0 to 2. After imputation, SNPs with $r^2 < 0.30$, an MAF $< 1\%$, or a Hardy-Weinberg
3 equilibrium $p < 1 \times 10^{-6}$ were removed. We retrieved genotypes of the SNPs for GRSs from the
4 imputed data for all study participants (**Supplemental Table S1 and Supplemental Table S2**).

5 **Leptin and Lipids measurement**

6 In the FHS, blood samples were collected after overnight fasting and analyzed following
7 standard protocols.⁴³ Serum leptin levels were determined by enzyme-linked immunosorbent
8 assay (ELISA) method at R&D Systems using the Quantikine Human Leptin Immunoassay.⁴³
9 Leptin was logarithmically transformed for analyses in the current study so that the data
10 distribution can meet the assumptions of linear regression models.

11 Fasting blood lipids, including TC, HDL-C, and TG, were measured using automated
12 enzymatic assays.⁴³ For participants taking lipid-lowering medications, TC was adjusted as
13 TC/0.8.⁴⁴ After adjustment, LDL-C was calculated using the Friedewald formula.⁴⁵ The adjusted
14 TC and LDL-C were used for analyses in the current study. Triglycerides were logarithmically
15 transformed (log-TG) in the current study so that the data distribution can meet the assumptions
16 of linear regression models.

17 **Covariates**

18 Demographic and health behavioral variables, including age, gender, education, smoking,
19 and drinking, were based on self-report. Education levels were categorized into “no more than
20 high school,” “some college,” and “bachelor’s degree or above.” Smoking was categorized into
21 “current smoker” or “not a current smoker” and drinking status into “current drinker” and “not a

1 current drinker.” Physical activity was measured with the physical activity index composite
2 score, which was calculated by summing the number of hours spent in each activity intensity
3 level weighted by their corresponding weight factor derived from the estimated oxygen
4 consumption requirement for each intensity level.⁴⁶ BMI was calculated as weight in kilograms
5 divided by the square of height in meters. Waist circumference was measured to next lower 1/4
6 inch by regional anthropometry.

7 **Statistical Analysis**

8 Weighted GRSs for leptin were calculated for each participant as the sum of the products
9 of the participant’s dosage scores for each SNP and the SNP’s estimated effect size. Since
10 obesity is highly associated with both leptin and blood lipids, our main focus was on GRS1, the
11 score generated using loci associated with leptin independent of BMI. The GRS1 for participants
12 was then categorized into quartiles. Means and standard deviations for continuous and
13 frequencies and percentages for categorical characteristics at baseline were calculated for each
14 quartile of the GRS1. *p* values for linear trends in those variables across quartiles of the GRS1
15 were estimated.

16 Three multivariate linear regression models were used to assess associations between log-
17 leptin and lipids, leptin GRS and log-leptin, and the leptin GRS and lipids, respectively. All
18 models were adjusted for age, sex, BMI, and waist circumference. To test robustness of the
19 leptin GRS and lipids associations, we additionally controlled for education, smoking, drinking,
20 and physical activity index score in the fully adjusted models. To explore whether associations
21 between the leptin GRS and lipids levels were modified by alcohol consumption, we performed
22 stratified analyses by drinking status. In each stratum of the drinking status, we tested

1 associations between leptin GRS and lipids by adjusting for age, sex, BMI, and waist
2 circumference in the base model and additionally adjusting for education, smoking, and physical
3 activity in the full model. Interactions between the leptin GRS and alcohol consumption were
4 tested among the overall participants by adding drinking and the interaction term, GRS×drinking,
5 to the models. All the above analyses were done for GRS1 and GRS2 separately. We quantified
6 the strength of the causal association of leptin with lipids using the instrumental variable
7 estimator.⁴⁷ The estimator was calculated as the ratio of the coefficient for leptin GRS and lipids
8 association to the coefficient for the leptin GRS and log-leptin association from the base models.

9 To rule out the effect of lipid-lowering medications, sensitivity analyses were performed
10 among those not taking lipid medication. To rule out the effect of both diabetes and lipid-
11 lowering medications, sensitivity analyses were performed among those not taking lipid- or
12 glucose-lowering medications. All analyses were performed using SAS software (version 9.4;
13 SAS Institute Inc., Cary, North Carolina). Two-sided *p* values were provided, and *p*<0.05 was
14 considered significant.

15 **Participant and Public Involvement**

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

18 **Results**

19 Characteristics of the study participants are presented in **Table 1**. Participants were on
20 average 40.2 years old at baseline. There were slightly more females (53.2%), and only 15.4%
21 had less than a high school education. The majority (89.1%) of the participants were current

1 drinkers, and 15.6% were current smokers. Participants were on average over weighted, with a
2 mean BMI of 26.9 kg/m² and mean waist girth of 36.6 inches. About 6.9% of the participants
3 were treated for dyslipidemia, and 1.9% were treated for diabetes. The BMI independent leptin
4 GRS1 was not associated with age ($p=0.23$), sex ($p=0.89$), education ($p=0.22$), smoking
5 ($p=0.53$), drinking ($p=0.32$), BMI ($p=0.94$), waist circumference ($p=0.70$), lipid-lowering
6 medication usage ($p=0.26$), or the physical activity index score ($p=0.51$), but with diabetes-
7 lowering medication usage ($p=0.03$). As expected, the GRS1 was positively associated with age,
8 sex, BMI, and waist circumference adjusted log-leptin ($p=4.56\times 10^{-5}$).

9 **BMI independent leptin GRS1 and blood lipids**

10 After controlling for age, sex, BMI, and waist circumference, log-leptin was positively
11 associated with TC ($\beta=8.56$, $p=6.35\times 10^{-18}$), LDL-C ($\beta=6.46$, $p=1.85\times 10^{-13}$), and log-TG ($\beta=0.13$,
12 $p=1.59\times 10^{-20}$), but was not associated with HDL-C ($\beta=-0.62$, $p=0.11$) (**Figure 1 and**
13 **Supplemental Figure S1**). Per unit increase in the leptin GRS1 was associated with a 1.21-unit
14 increase in the age, sex, BMI, and waist circumference adjusted log-leptin ($p=4.56\times 10^{-5}$). The
15 leptin GRS1 was inversely associated with age, sex, BMI, and waist circumference adjusted log-
16 TG ($\beta=-0.66$, $p=0.01$) (**Figure 1**). When further adjusting for education, smoking, drinking, and
17 physical activity, the GRS1 and log-TG association was still significant ($\beta=-0.69$, $p=0.008$,
18 **Table 2**). Instrumental variable estimation indicated that log-TG levels decreased by 0.55 (95%
19 CI: 0.05, 1.00, $p=0.02$) per unit increase of genetically determined log-leptin level (**Figure 1**).
20 The leptin GRS1 was inversely associated with TC ($\beta=-12.50$, $p=0.49$) and LDL-C ($\beta=-0.11$,
21 $p=0.99$) and positively associated with HDL-C ($\beta=5.42$, $p=0.44$), however, the correlations were

1 not significant. The GRS1 and blood lipids associations were not modified by drinking status
2 (Table 2).

3 **BMI dependent leptin GRS2 and blood lipids**

4 As expected, the BMI dependent leptin GRS2 was not associated with any covariate
5 except for the BMI ($p=0.02$) and waist circumference ($p=0.03$). In the analyses controlling for
6 age, sex, BMI, and waist circumference, the GRS2 was significantly associated with lower levels
7 of log-TG ($p=0.009$) and nominally associated with lower levels of HDL-C ($p=0.09$) and TC
8 ($p=0.08$) (Supplemental Figure S2). When stratified by drinking status, the leptin GRS2 was
9 negatively associated with LDL-C ($\beta=-92.51$, $p=0.03$), log-TG ($\beta=-2.07$, $p=0.004$), and TC ($\beta=-$
10 144.68 , $p=0.003$) only among non-current drinkers (Table 3). When further adjusting for
11 education, smoking, drinking, and physical activity, those associations persisted (Table 3).
12 Furthermore, significant interactions between leptin GRS2 and alcohol drinking were identified
13 for LDL-C ($p=0.03$), log-TG ($p=0.03$), and TC ($p=0.02$) (Table 3).

14 When restricting to participants not taking lipid-lowering medication and those not taking
15 lipid- or glucose-lowering medications, respectively, the associations of GRS1 and GRS2 with
16 blood lipids were similar to those as shown above (Supplemental Table S3, S4, S5 and S6).

17 **Discussion**

18 To the best of our knowledge, the current study is the first Mendelian randomization
19 analysis on leptin and blood lipids. We provide robust evidence to support a potentially causal
20 relation between leptin and reduced levels of triglycerides among a majority of overweight and
21 obese population of European ancestry. Furthermore, we demonstrated that alcohol consumption

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3 1 modified the association of BMI dependent GRS2 with lipids in that genetically determined
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5 2 leptin levels were inversely associated with LDL-C, log-TG, and TC, but only among individuals
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8 3 who were not current drinkers.
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11 4 Both the BMI dependent- and independent- GRSs were associated with lower level of
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13 5 log-TG in the current study. Inconsistent associations between leptin and blood lipids have been
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15 6 observed in previous studies. In a small study of 80 postmenopausal women, serum leptin was
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17 7 positively associated with HDL-C, TG, and TC, and inversely associated with LDL-C.⁴⁸ Another
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19 8 study conducted with 294 healthy school children reported that leptin was only associated with
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21 9 increased TG.⁴⁹ However, a study of 476 residents from Cameroon reported a positive
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23 10 correlation between leptin, LDL-C, and TC, and a positive association between leptin and TC,
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25 11 but no association between leptin and HDL-C or TG.⁵⁰ In a more recent study of 134 physically
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27 12 active postmenopausal women, no significant correlation was detected for leptin and blood
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29 13 lipids.⁵¹ The divergent results of previous studies make it impossible to infer a relationship
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31 14 between leptin and blood lipids. Possible reasons for the divergent findings include varying
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33 15 sample sizes, failure to account for residual and unmeasured confounding, and the genetic
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35 16 background of the study population. Through Mendelian randomization analyses, we
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37 17 demonstrated that genetically determined leptin was inversely associated with log-TG. It is well
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39 18 known that alleles, such as risk alleles for leptin, are randomly assigned at meiosis and therefore,
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41 19 are independent of non-genetic confounders. The association between leptin GRS and log-TG in
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43 20 the current study was less prone to confounding. This also highlights the importance of using
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45 21 Mendelian randomization to delineate causal relationships. Our finding is further supported by
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47 22 previous physiologic studies, among which, leptin was demonstrated to inhibit lipogenesis,
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49 23 stimulate lipolysis, and reduce triglyceride uptake.⁵² However, the association of HDL-C and TC
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1 were only nominally significant with BMI dependent GRS2 in the current study. It could be due
2 to lack of statistical power or existing interaction of leptin and drinking. Therefore, we cannot
3 rule out causal relationships between leptin and those lipid measures. Future large-scale
4 Mendelian randomization studies are warranted to evaluate associations of leptin GRS with
5 HDL-C, LDL-C, and TC.

6 The BMI independent GRS1 was only associated with log-TG, while the BMI dependent
7 GRS2 was also in nominal associations with HDL-C and TC. In addition, alcohol drinking
8 modified the GRS2-lipids associations but not the GRS1-lipids associations. This indicated that
9 the role of leptin in blood lipids regulation may be through multiple mechanisms. The BMI
10 dependent GRS2 were inversely associated with LDL-C, log-TG, and TC only among non-
11 current drinkers, but not among current drinkers. Although future studies are warranted to
12 confirm these interactions, previous physiological studies may provide a reasonable explanation.
13 Singh and colleagues demonstrated that the increased expression of caveolin-1 impairs leptin
14 signaling and attenuates leptin-dependent effect to prevent lipid accumulation in human white
15 pre-adipocytes.⁵³ Meanwhile, Caveolin-1 can be increased by alcohol drinking.⁵⁴

16 Our study represents the first Mendelian randomization analyses for leptin and blood
17 lipids in a population of European ancestry. A major strength of this study is the stringent quality
18 control methods used in measuring genotypes, phenotype, and covariates in the FHS 3rd
19 Generation Cohort. Those methods can reduce measurement error and increase the statistical
20 power needed to identify associations between leptin GRS and lipids. We also identify some
21 limitations. First, pleiotropy effects of SNPs included in the leptin GRS may confound the leptin
22 GRS and lipids associations. It is possible that our results may represent a shared genetic basis

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3 1 between leptin and lipids rather than a causal relationship. Second, we may not have sufficient
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5 2 power to detect associations between genetically determined leptin levels and LDL-C, HDL-C,
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7 3 and TC. Larger Mendelian randomization studies are warranted to evaluate associations between
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9 4 leptin and LDL-C, HDL-C, and TC. Third, we did not control for total energy intake in our
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11 5 analyses because food frequency questionnaire survey was not conducted in the third generation
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13 6 cohort at baseline when leptin was measured. However, leptin combines with receptors in the
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15 7 hypothalamus to reduce appetite and increase energy expenditure. Therefore, total energy intake
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17 8 is in the pathway from leptin to lipids metabolism and may not meet the criteria of being a
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19 9 confounder. Forth, the type of alcohol consumed was not measured and cannot be considered in
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21 10 the current analyses. It is possible that the alcohol consumed in the studied population is mainly
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23 11 wine and/or beers, which contain high level of resveratrol and phytochemical. The two chemicals
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25 12 may benefit lipid metabolism.^{55 56} However, the two chemicals do not share similar genetic
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27 13 profile with leptin, and consequently, they should not be correlated with leptin and cannot affect
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29 14 the associations between leptin GRS and blood lipids. Fifth, genetically determined ratio of
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31 15 leptin to leptin receptor may be a better measure to study the role of leptin in lipid metabolism.
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33 16 However, we could not find a genome-wide study on the ratio of leptin to leptin receptor,
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35 17 therefore, a GRS on the ratio cannot be calculated. Future genome-wide studies on the ratio of
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37 18 leptin to leptin receptor are warranted. Finally, our analyses were restricted to individuals of
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39 19 European ancestry. Our findings may not be generalizable to populations of other ancestries.

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48 20 In summary, the present study provided robust evidence for a potential causal effect of
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50 21 leptin on reduced triglycerides. In addition, genetically determined leptin may regulate blood
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52 22 lipids through different mechanisms, and the association between leptin and lipid metabolism
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54 23 may be modified by alcohol consumption.

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3 **Author Contributions**

4 Conceptualization, Changwei Li, José Cordero, Jia-Sheng Wang, Shengxu Li, and Zhi-
5 Yong Zou; Formal analysis, Luqi Shen and Ye Shen; Supervision, Changwei Li, José Cordero,
6 Jia-Sheng Wang, Shengxu Li, and Zhi-Yong-Zou; Writing – original draft, Luqi Shen; Writing –
7 review & editing, Changwei Li, José Cordero, Luqi Shen, Lirong Liang, and Zhi-Yong Zou.

8 **Conflict of interest**

9 Conflicts of interest and disclosures: none.

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13 **Data sharing statement**

14 The deidentified dataset supporting this study is available on the database of genotype
15 and phenotype (dbGaP) at the National Center for Biotechnology Information (NCBI).
16 https://www.ncbi.nlm.nih.gov/projects/gap/cgi-bin/study.cgi?study_id=phs000007.v30.p11. The
17 researchers are able to reuse the dataset on the condition that they get the approval from dbGaP
18 and their institution.

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Figure legends

Figure 1. The relationship between leptin, genetic risk score for leptin and triglycerides (TG) in Framingham Heart Study the 3rd Generation cohort.

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Table 1. Characteristics of the Study Participants by Genetic Risk Score 1 (GRS1)^a for logarithmically transformed Leptin in Framingham Heart Study 3rd Generation Cohort.

Covariates	Overall	Quartiles of the leptin GRS				P
	(n=3,860)	Q1 (n=964)	Q2 (n=961)	Q3 (n=977)	Q4 (n=958)	
Genetic risk score, 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 (%)						
<i>No more than high school</i>	591 (15.4)	141 (14.7)	146 (15.3)	157 (16.1)	147 (15.4)	
<i>Some college</i>	1213 (31.5)	306 (31.8)	287 (30.0)	313 (32.1)	307 (32.3)	0.22
<i>Bachelor's degree and above</i>	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
Low Density Lipoprotein, 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
High Density Lipoprotein, 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
Triglycerides, 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*
Total Cholesterol, 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

BMI=body mass index; Log-leptin=logarithmically transformed leptin; GRS=Genetic Risk Score; SD=standard deviation.

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3^a Genetic risk scores 1(GRS1) for leptin was generated by summing leptin increasing alleles of 3 SNPs adjusted for BMI, weighted by
4 their corresponding effect sizes reported by Kilpelainen et al.

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6 *Log transformed leptin and triglycerides were used to calculate the *P*-values.
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Table 2. Association of BMI independent Leptin GRS1^a with Baseline Lipids among Overall, Drinking, and Non-Drinking Participants of the Framingham Heart Study 3rd Generation Cohort, Respectively.

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

GRS=Genetic Risk Score; HDL-C =High-density lipoprotein cholesterol; LDL-C =Low-density lipoprotein cholesterol; log-TG=logarithmically transformed triglycerides; SE=standard error.

^a Genetic risk scores1 (GRS1) for leptin was generated by summing leptin increasing alleles of 3 SNPs adjusted for body mass index (BMI), weighted by their corresponding effect sizes reported by Kilpelainen et al;

^b 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;

^c Adjusted for age, sex, education, smoking, drinking, BMI, waist, and physical activity among the overall sample;

^d Assessed by adding an interaction term, drinking×GRS to the fully adjusted model among the overall participants.

Table 3. Association of BMI dependent Leptin GRS^{2a} with Baseline Lipids among Overall, Drinking, and Non-Drinking Participants of the Framingham Heart Study 3rd Generation Cohort, Respectively.

	Age, sex, BMI, waist adjusted model		$P_{\text{interaction}}^b$	Fully adjusted model ^c		$P_{\text{interaction}}^d$
	Beta(SE)	P		Beta(SE)	P	
HDL-C						
Overall	-10.67 (6.20)	0.09		-10.98 (6.22)	0.08	
Not current drinkers	-0.69 (16.31)	0.97	0.56	0.82 (16.55)	0.96	0.52
Current drinkers	-12.15 (6.64)	0.07		-11.94 (6.68)	0.07	
LDL-C						
Overall	-2.11 (14.05)	0.88		-2.81 (14.21)	0.84	
Not current drinkers	-92.51 (43.02)	0.03	0.03	-101.15 (43.78)	0.02	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		-0.59 (0.23)	0.01	
Not current drinkers	-2.07 (0.71)	0.004	0.03	-2.03 (0.72)	0.005	0.03
Current drinkers	-0.40 (0.24)	0.09		-0.42 (0.24)	0.08	
Total cholesterol						
Overall	-28.05 (15.91)	0.08		-28.74 (16.02)	0.07	
Not current drinkers	-144.68 (47.61)	0.003	0.02	-151.32 (48.37)	0.002	0.01
Current drinkers	-13.19 (16.90)	0.44		-14.67 (16.98)	0.39	

GRS=Genetic Risk Score; HDL-C =High-density lipoprotein cholesterol; LDL-C =Low-density lipoprotein cholesterol; log-TG=logarithmically transformed triglycerides; SE=standard error.

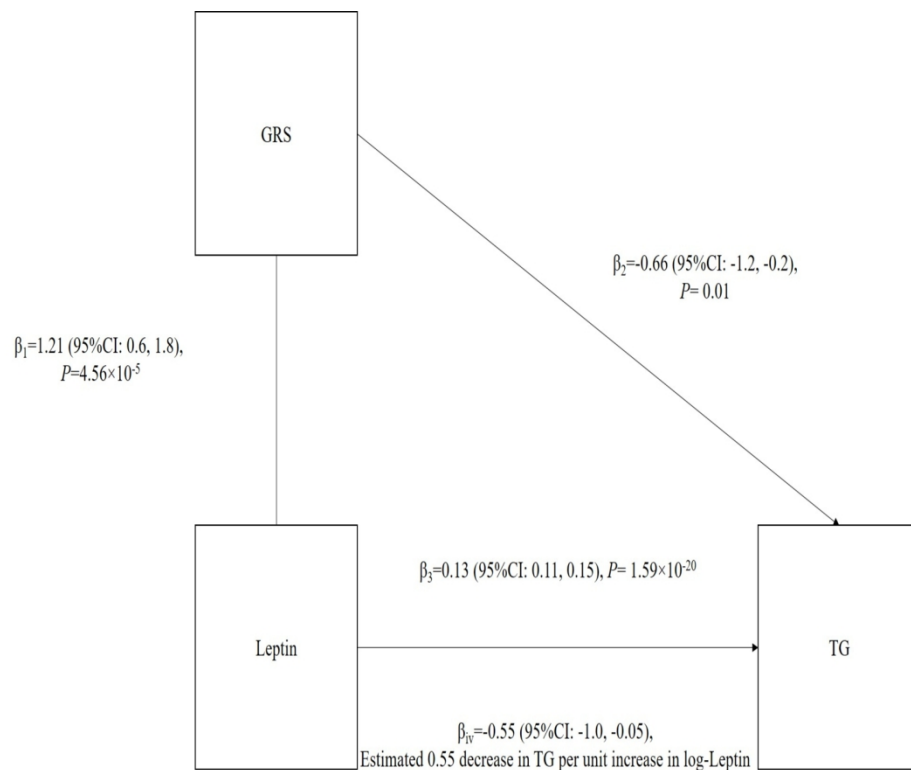
^a Genetic risk scores² (GRS²) for leptin was generated by summing leptin increasing alleles of 3 SNPs unadjusted for body mass index (BMI), weighted by their corresponding effect sizes reported by Kilpelainen et al;

^b 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;

^c Adjusted for age, sex, education, smoking, drinking, BMI, waist, and physical activity among the overall sample;

^d Assessed by adding an interaction term, drinking×GRS to the fully adjusted model among the overall participants.

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Supplemental Table S1. Basic Information of three SNPs for BMI independent leptin GRS1^a reaching genome-wide significance ($P < 5 \times 10^{-8}$)

Chromosome		Coded	Non-coded	Nearest			
Position	rsID	Allele	Allele	R ²	Function	Gene	Population
2:27742603	rs780093	C	T	0.995	intron variant	GCKR	European American
7:127860163	rs10487505	G	C	0.989	intron variant	LEP	European American
20:37333012	rs6071166	C	A	0.973	intergenic	SLC32A1	European American

BMI=body mass index

^a Genetic risk scores 1 (GRS1) for leptin was generated by summing leptin increasing alleles of 3 SNPs adjusted for body mass index (BMI), weighted by their corresponding effect sizes reported by Kilpelainen et al.

Supplemental Table S2. Basic Information of three SNPs for BMI dependent leptin GRS2^a reaching genome-wide significance (P<5×10⁻⁸)

Chromosome		Coded	Non-coded	Nearest			
Position	rsID	Allele	Allele	R ²	Function	Gene	Population
2:27742603	rs780093	C	T	0.995	intron variant	GCKR	European American
3:156798775	rs900400	T	C	0.691	upstream variant 2KB	CCNL1	European American
16:53813450	rs8043757	A	T	0.999	intron variant	FTO	European American

BMI=body mass index

^a Genetic risk scores 2 (GRS2) for leptin was generated by summing leptin increasing alleles of 3 SNPs unadjusted for body mass index (BMI), weighted by their corresponding effect sizes reported by Kilpelainen et al.

Supplemental Table S3. Association of BMI independent Leptin GRS1^a with Baseline Lipids among Overall, Drinking, and Non-Drinking Participants without treating for Lipids of the Framingham Heart Study 3rd Generation Cohort, Respectively.

	Age, sex, BMI, waist adjusted model		<i>P</i> _{interaction^b}	Fully adjusted model ^c		<i>P</i> _{interaction^d}
	Beta(SE)	<i>P</i>		Beta(SE)	<i>P</i>	
HDL-C						
Overall	5.25 (7.44)	0.48		7.59 (7.47)	0.31	
Not current drinkers	18.83 (19.59)	0.34	0.86	20.43 (20.23)	0.31	0.83
Current drinkers	6.22 (7.97)	0.44		7.19 (8)	0.37	
LDL-C						
Overall	14.86 (16.06)	0.35		13.43 (16.2)	0.41	
Not current drinkers	36.1 (49.22)	0.46	0.51	27.18 (50.69)	0.59	0.61
Current drinkers	12.19 (17.05)	0.47		10.48 (17.11)	0.54	
log-TG						
Overall	-0.69 (0.27)	0.01		-0.72 (0.27)	0.007	
Not current drinkers	-1.72 (0.84)	0.04	0.20	-1.61 (0.86)	0.06	0.21
Current drinkers	-0.58 (0.28)	0.04		-0.6 (0.28)	0.03	

Total cholesterol

Overall	-0.09 (18.19)	1.00		-0.67 (18.26)	0.97	
Not current drinkers	3.78 (55.05)	0.95	0.94	-2.29 (56.42)	0.97	0.97
Current drinkers	0.98 (19.29)	0.96		-0.34 (19.32)	0.99	

BMI=body mass index; GRS=Genetic Risk Score; HDL-C=High-density lipoprotein cholesterol; LDL-C=Low-density lipoprotein cholesterol; log-TG=logarithmically transformed triglycerides; SE=standard error

^a Genetic risk scores 1 (GRS1) for leptin was generated by summing leptin increasing alleles of 3 SNPs adjusted for body mass index (BMI), weighted by their corresponding effect sizes reported by Kilpelainen et al;

^b 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;

^c Adjusted for age, sex, education, smoking, drinking, BMI, waist, and physical activity among the overall sample;

^d Assessed by adding an interaction term, drinking×GRS to the fully adjusted model among the overall participants.

Supplemental Table S4. Association of BMI dependent Leptin GRS2^a with Baseline Lipids among Overall, Drinking, and Non-Drinking Participants without treating for Lipids of the Framingham Heart Study 3rd Generation Cohort, Respectively.

	Age, sex, BMI, waist adjusted model			Fully adjusted model ^c		
	Beta(SE)	<i>P</i>	<i>P</i> _{interaction} ^b	Beta(SE)	<i>P</i>	<i>P</i> _{interaction} ^d
HDL-C						
Overall	-9.73 (6.50)	0.13		-9.66 (6.53)	0.14	
Not current drinkers	-2.61 (17.44)	0.88	0.72	-1.22 (17.77)	0.95	0.68
Current drinkers	-10.52 (6.94)	0.13		-10.19 (6.99)	0.15	
LDL-C						
Overall	-0.42 (14.02)	0.98		-1.56 (14.17)	0.91	
Not current drinkers	-83.19 (43.58)	0.06	0.07	-94.12 (44.22)	0.03	0.05
Current drinkers	9.71 (14.86)	0.51		7.61 (14.96)	0.61	
log-TG						
Overall	-0.63 (0.23)	0.007		-0.63 (0.23)	0.007	
Not current drinkers	-2.09 (0.74)	0.005	0.04	-2.00 (0.75)	0.008	0.05
Current drinkers	-0.45 (0.25)	0.07		-0.47 (0.25)	0.05	
Total cholesterol						

Overall	-25.28 (15.87)	0.11		-26.38 (15.97)	0.10	
Not current drinkers	-136.15 (48.44)	0.005	0.03	-144.29 (48.92)	0.003	0.02
Current drinkers	-10.96 (16.82)	0.51		-13.64 (16.90)	0.42	

BMI=body mass index; GRS=Genetic Risk Score; HDL-C=High-density lipoprotein cholesterol; LDL-C=Low-density lipoprotein cholesterol; log-TG=logarithmically transformed triglycerides; SE=standard error

^a Genetic risk scores 2 (GRS2) for leptin was generated by summing leptin increasing alleles of 3 SNPs unadjusted for body mass index (BMI), weighted by their corresponding effect sizes reported by Kilpelainen et al;

^b 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;

^c Adjusted for age, sex, education, smoking, drinking, BMI, waist, and physical activity among the overall sample;

^d Assessed by adding an interaction term, drinking×GRS to the fully adjusted model among the overall participants.

Supplemental Table S5. Association of BMI independent Leptin GRS1^a with Baseline Lipids among Overall, Drinking, and Non-Drinking Participants without treating for Lipids and Diabetes of the Framingham Heart Study 3rd Generation Cohort, Respectively.

	Age, sex, BMI, waist adjusted model			Fully adjusted model ^c		
	Beta(SE)	<i>P</i>	<i>P</i> _{interaction} ^b	Beta(SE)	<i>P</i>	<i>P</i> _{interaction} ^d
HDL-C						
Overall	5.64 (7.47)	0.45		8.00 (7.49)	0.29	
Not current drinkers	20.89 (19.62)	0.29	0.72	22.51 (20.25)	0.27	0.69
Current drinkers	6.22 (7.98)	0.44		7.17 (8.02)	0.37	
LDL-C						
Overall	15.29 (16.11)	0.34		13.42 (16.27)	0.41	
Not current drinkers	33.51 (49.77)	0.50	0.58	24.63 (51.26)	0.63	0.68
Current drinkers	12.68 (17.09)	0.46		10.99 (17.16)	0.52	
log-TG						
Overall	-0.66 (0.27)	0.01		-0.69 (0.27)	0.01	
Not current drinkers	-1.60 (0.85)	0.06	0.23	-1.46 (0.86)	0.09	0.25
Current drinkers	-0.57 (0.28)	0.04		-0.59 (0.28)	0.04	

Total cholesterol

Overall	1.17 (18.24)	0.95		0.14 (18.33)	0.99	
Not current drinkers	6.19 (55.64)	0.91	0.93	1.04 (57.03)	0.99	0.99
Current drinkers	1.56 (19.35)	0.94		0.26 (19.38)	0.99	

BMI=body mass index; GRS=Genetic Risk Score; HDL-C =High-density lipoprotein cholesterol; LDL-C =Low-density lipoprotein cholesterol; log-TG=logarithmically transformed triglycerides; SE=standard error

^a Genetic risk scores 1 (GRS1) for leptin was generated by summing leptin increasing alleles of 3 SNPs adjusted for body mass index (BMI), weighted by their corresponding effect sizes reported by Kilpelainen et al;

^b 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;

^c Adjusted for age, sex, education, smoking, drinking, BMI, waist, and physical activity among the overall sample;

^d Assessed by adding an interaction term, drinking×GRS to the fully adjusted model among the overall participants.

Supplemental Table S6. Association of BMI dependent Leptin GRS2^a with Baseline Lipids among Overall, Drinking, and Non-Drinking Participants without treating for Lipids and Diabetes of the Framingham Heart Study 3rd Generation Cohort, respectively.

	Age, sex, BMI, waist adjusted model		<i>P</i> _{interaction^b}	Fully adjusted model ^c		<i>P</i> _{interaction^d}
	Beta(SE)	<i>P</i>		Beta(SE)	<i>P</i>	
HDL-C						
Overall	-10.39 (6.52)	0.11		-10.48 (6.55)	0.11	
Not current drinkers	-8.18 (17.54)	0.64	0.95	-7.77 (17.87)	0.66	0.92
Current drinkers	-10.58 (6.96)	0.13		-10.32 (7.01)	0.14	
LDL-C						
Overall	-1.58 (14.07)	0.91		-3.3 (14.22)	0.82	
Not current drinkers	-95.04 (44.19)	0.03	0.04	-105.66 (44.81)	0.02	0.03
Current drinkers	8.84 (14.90)	0.55		6.84 (15.00)	0.65	
log-TG						
Overall	-0.62 (0.23)	0.008		-0.62 (0.23)	0.008	
Not current drinkers	-2.00 (0.75)	0.008	0.06	-1.87 (0.76)	0.01	0.08

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3	Current drinkers	-0.45 (0.25)	0.07		-0.48 (0.25)	0.05	
4							
5	Total cholesterol						
6							
7	Overall	-26.72 (15.92)	0.09		-28.67 (16.03)	0.07	
8							
9	Not current drinkers	-150.52 (49.04)	0.002	0.01	-158.43 (49.51)	0.002	0.01
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11	Current drinkers	-12.06 (16.87)	0.47		-14.69 (16.95)	0.39	
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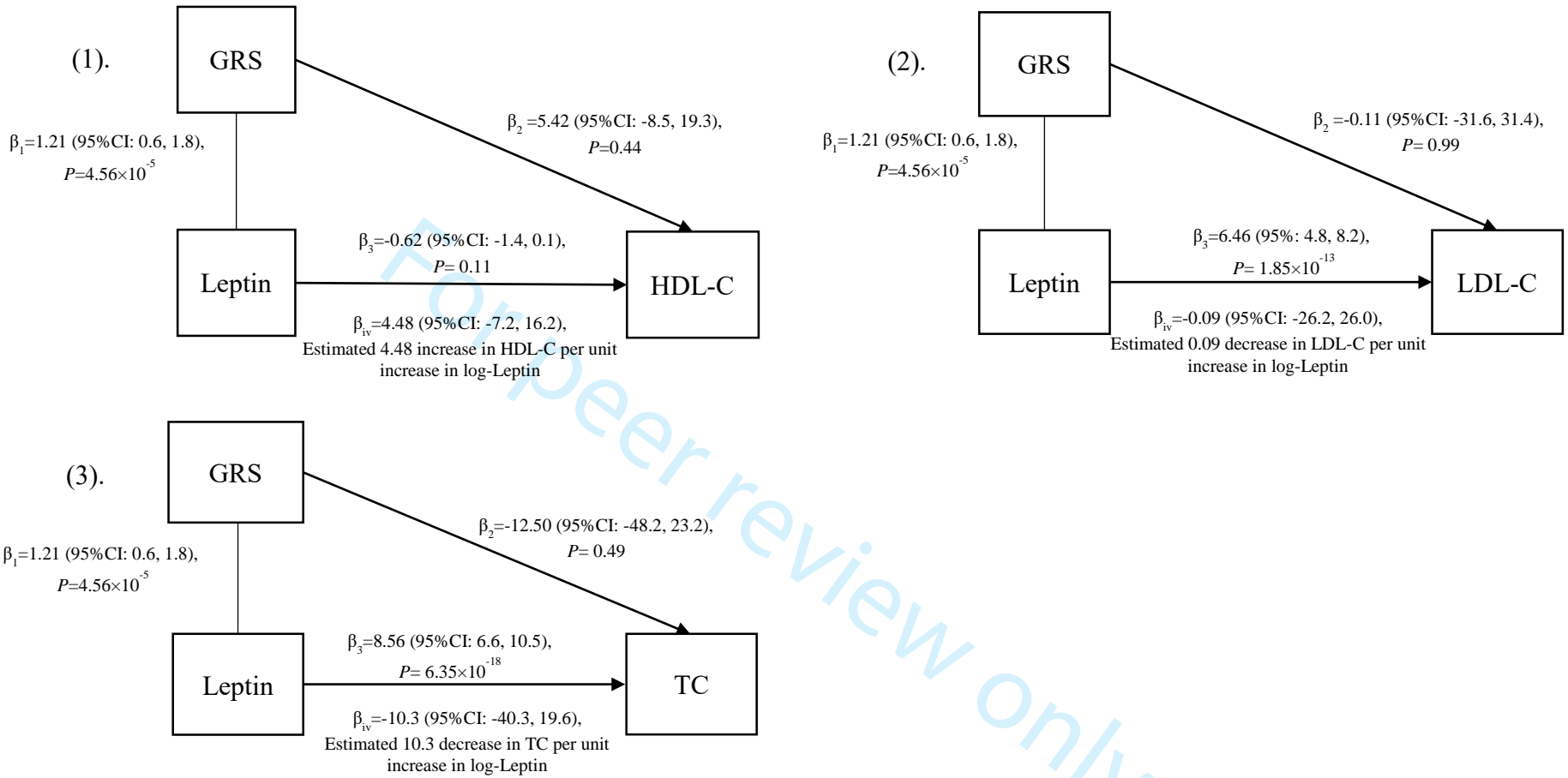
BMI=body mass index; GRS=Genetic Risk Score; HDL-C=High-density lipoprotein cholesterol; LDL-C=Low-density lipoprotein cholesterol; log-TG=logarithmically transformed triglycerides; SE=standard error

^a Genetic risk scores 2 (GRS2) for leptin was generated by summing leptin increasing alleles of 3 SNPs unadjusted for body mass index (BMI), weighted by their corresponding effect sizes reported by Kilpelainen et al;

^b 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;

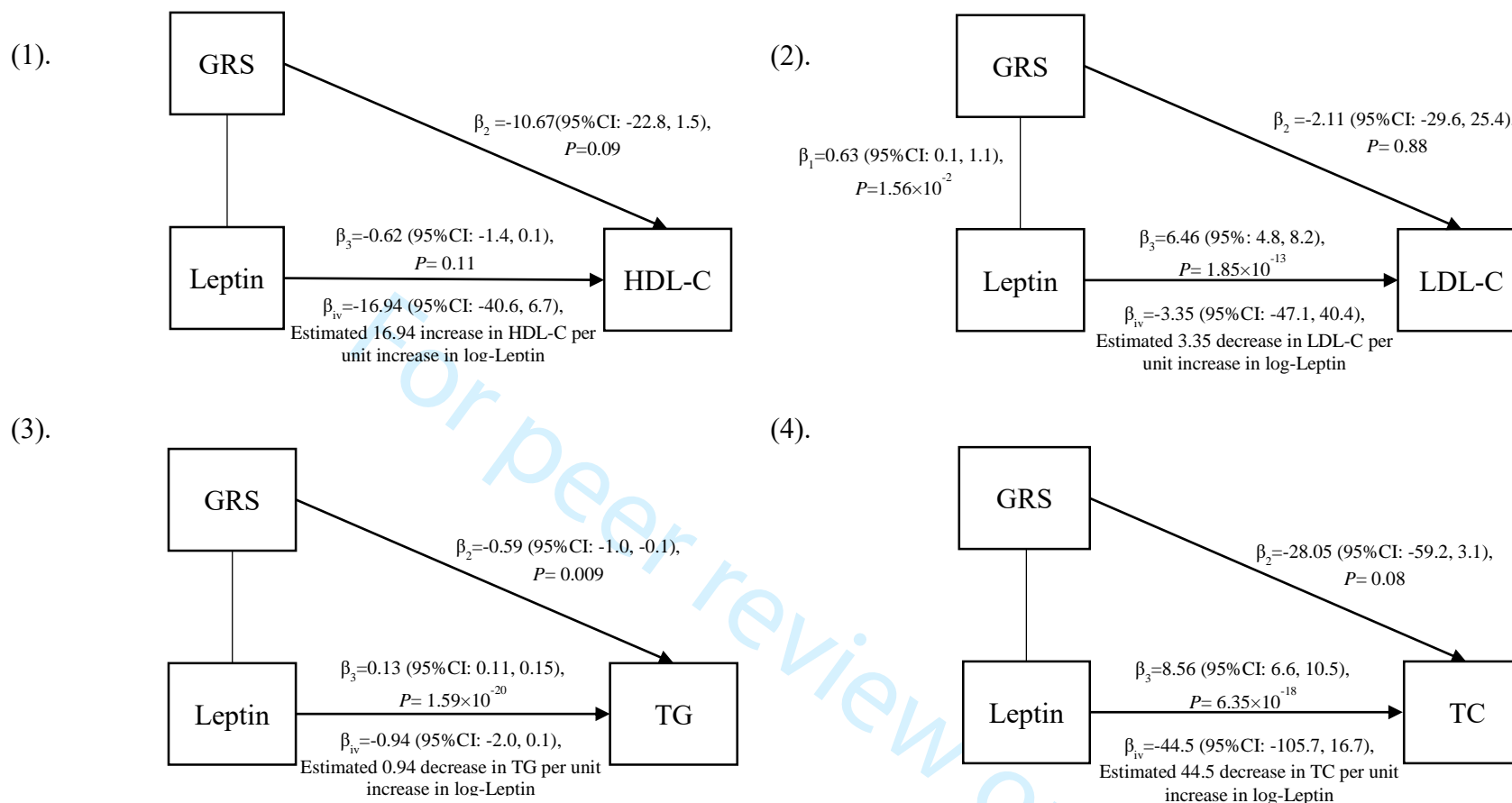
^c Adjusted for age, sex, education, smoking, drinking, BMI, waist, and physical activity among the overall sample;

^d Assessed by adding an interaction term, drinking×GRS to the fully adjusted model among the overall participants.



Supplemental Figure S1. The relationship between Leptin, Genetic Risk Score 1 (GRS1) for Leptin and Lipids in Framingham Heart Study the 3rd Generation cohort.

1. The relationship between leptin, genetic risk score for leptin and high density lipoprotein cholesterol (HDL-C)
2. The relationship between leptin, genetic risk score for leptin and low density lipoprotein cholesterol (LDL-C)
3. The relationship between leptin, genetic risk score for leptin and total cholesterol (TC)



32 **Supplemental Figure S2. The relationship between Leptin, Genetic Risk Score 2 (GRS2) for Leptin and Lipids in Framingham**
33 **Heart Study the 3rd Generation cohort.**

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- 36 1. The relationship between leptin, genetic risk score for leptin and high density lipoprotein cholesterol (HDL-C)
 - 37 2. The relationship between leptin, genetic risk score for leptin and low density lipoprotein cholesterol (LDL-C)
 - 38 3. The relationship between leptin, genetic risk score for leptin and triglycerides (TG)
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4. The relationship between leptin, genetic risk score for leptin and total cholesterol (TC)

For peer review only

Reporting checklist for genetic association study.

Based on the STREGA guidelines.

Instructions to authors

Complete this checklist by entering the page numbers from your manuscript where readers will find each of the items listed below.

Your article may not currently address all the items on the checklist. Please modify your text to include the missing information. If you are certain that an item does not apply, please write "n/a" and provide a short explanation.

Upload your completed checklist as an extra file when you submit to a journal.

In your methods section, say that you used the STREGA reporting guidelines, and cite them as:

Little J, Higgins JP, Ioannidis JP, Moher D, Gagnon F, von Elm E, Khoury MJ, Cohen B, Davey-Smith G, Grimshaw J, Scheet P, Gwinn M, Williamson RE, Zou GY, Hutchings K, Johnson CY, Tait V, Wiens M, Golding J, van Duijn C, McLaughlin J, Paterson A, Wells G, Fortier I, Freedman M, Zecevic M, King R, Infante-Rivard C, Stewart A, Birkett N; STrengthening the REporting of Genetic Association Studies. STrengthening the REporting of Genetic Association Studies (STREGA): An Extension of the STROBE Statement.

		Reporting Item	Page Number
Title	#1a	Indicate the study's design with a commonly used term in the title or the abstract	1
Abstract	#1b	Provide in the abstract an informative and balanced summary of what was done and what was found	2
	#2	Explain the scientific background and rationale for the investigation being reported	4
	#3	State specific objectives, including any prespecified hypotheses. State if the study is the first report of a genetic association, a replication effort, or both.	5
	#4	Present key elements of study design early in the paper	5
	#5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	5-6

1	#6a	Cohort study – Give the eligibility criteria, and the sources and methods	5-6
2		of selection of participants. Describe methods of follow-up. Case-control	
3		study – Give the eligibility criteria, and the sources and methods of case	
4		ascertainment and control selection. Give the rationale for the choice of	
5		cases and controls. Cross-sectional study – Give the eligibility criteria,	
6		and the sources and methods of selection of participants. Give	
7		information on the criteria and methods for selection of subsets of	
8		participants from a larger study, when relevant.	
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14	#6b	Cohort study – For matched studies, give matching criteria and number	n/a
15		of exposed and unexposed. Case-control study – For matched studies,	
16		give matching criteria and the number of controls per case.	
17			
18			
19	#7a	Clearly define all outcomes, exposures, predictors, potential	7-8
20		confounders, and effect modifiers. Give diagnostic criteria, if applicable	
21			
22			
23	#7b	Clearly define genetic exposures (genetic variants) using a widely-used	6-7
24		nomenclature system. Identify variables likely to be associated with	
25		population stratification (confounding by ethnic origin).	
26			
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28	#8a	For each variable of interest give sources of data and details of methods	5-6
29		of assessment (measurement). Describe comparability of assessment	
30		methods if there is more than one group. Give information separately for	
31		for exposed and unexposed groups if applicable.	
32			
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35	#8b	Describe laboratory methods, including source and storage of DNA,	6-7
36		genotyping methods and platforms (including the allele calling algorithm	
37		used, and its version), error rates and call rates. State the laboratory /	
38		centre where genotyping was done. Describe comparability of laboratory	
39		methods if there is more than one group. Specify whether genotypes	
40		were assigned using all of the data from the study simultaneously or in	
41		smaller batches.	
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46	#9a	Describe any efforts to address potential sources of bias	8-9
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48	#9b	Describe any efforts to address potential sources of bias	8-9
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51	#10	Explain how the study size was arrived at	5-6
52			
53	#11	Explain how quantitative variables were handled in the analyses. If	6
54		applicable, describe which groupings were chosen, and why. If	
55		applicable, describe how effects of treatment were dealt with.	
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59	#12a	Describe all statistical methods, including those used to control for	8-9
60		For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml	

1	confounding. State software version used and options (or settings)	
2	chosen.	
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4	#12b Describe any methods used to examine subgroups and interactions	9
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6	#12c Explain how missing data were addressed	9
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8	#12d If applicable, explain how loss to follow-up was addressed	n/a
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11	#12e Describe any sensitivity analyses	9
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13	#12f State whether Hardy-Weinberg equilibrium was considered and, if so,	7
14	how.	
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17	#12g Describe any methods used for inferring genotypes or haplotypes	7
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19	#12h Describe any methods used to assess or address population	7
20	stratification.	
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23	#12i Describe any methods used to address multiple comparisons or to	7
24	control risk of false positive findings.	
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27	#12j Describe any methods used to address and correct for relatedness	n/a
28	among subjects	
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31	#13a Report numbers of individuals at each stage of study—eg numbers	5-6
32	potentially eligible, examined for eligibility, confirmed eligible, included in	
33	the study, completing follow-up, and analysed. Give information	
34	separately for for exposed and unexposed groups if applicable. Report	
35	numbers of individuals in whom genotyping was attempted and numbers	
36	of individuals in whom genotyping was successful.	
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41	#13b Give reasons for non-participation at each stage	n/a
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43	#13c Consider use of a flow diagram	n/a
44		
45	#14a Give characteristics of study participants (eg demographic, clinical,	9-10
46	social) and information on exposures and potential confounders. Give	
47	information separately for exposed and unexposed groups if applicable.	
48	Consider giving information by genotype	
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52	#14b Indicate number of participants with missing data for each variable of	9-10
53	interest	
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56	#14c Cohort study – Summarize follow-up time, e.g. average and total	n/a
57	amount.	
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- 1 #15 Cohort study Report numbers of outcome events or summary measures 9/10
 2 over time. Give information separately for exposed and unexposed
 3 groups if applicable. Report outcomes (phenotypes) for each genotype
 4 category over time Case-control study – Report numbers in each
 5 exposure category, or summary measures of exposure. Give information
 6 separately for cases and controls . Report numbers in each genotype
 7 category. Cross-sectional study – Report numbers of outcome events or
 8 summary measures. Give information separately for exposed and
 9 unexposed groups if applicable. Report outcomes (phenotypes) for each
 10 genotype category
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 17 #16a Give unadjusted estimates and, if applicable, confounder-adjusted 10-11
 18 estimates and their precision (eg, 95% confidence interval). Make clear
 19 which confounders were adjusted for and why they were included
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 22 #16b Report category boundaries when continuous variables were categorized 10-11
 23
 24 #16c If relevant, consider translating estimates of relative risk into absolute 10-11
 25 risk for a meaningful time period
 26
 27
 28 #16d Report results of any adjustments for multiple comparisons 10-11
 29
 30 #17a Report other analyses done—e.g., analyses of subgroups and 10-11
 31 interactions, and sensitivity analyses
 32
 33 #17b Report other analyses done—e.g., analyses of subgroups and 10-11
 34 interactions, and sensitivity analyses
 35
 36 #17c Report other analyses done—e.g., analyses of subgroups and 10-11
 37 interactions, and sensitivity analyses
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 40 #18 Summarise key results with reference to study objectives 11-13
 41
 42
 43 #19 Discuss limitations of the study, taking into account sources of potential 13-14
 44 bias or imprecision. Discuss both direction and magnitude of any
 45 potential bias.
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 48 #20 Give a cautious overall interpretation considering objectives, limitations, 14
 49 multiplicity of analyses, results from similar studies, and other relevant
 50 evidence.
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 53 #21 Discuss the generalisability (external validity) of the study results 14
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 56 #22 Give the source of funding and the role of the funders for the present 15
 57
 58
 59

1 study and, if applicable, for the original study on which the present article
2 is based
3

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5 CC-BY. This checklist was completed on 22. September 2018 using <http://www.goodreports.org/>, a
6 tool made by the [EQUATOR Network](#) in collaboration with [Penelope.ai](#)
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