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

Running title: Genetically determined leptin and lipids

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Abbreviations: CVD, cardiovascular disease; dbGaP, the database of genotype and phenotype; FHS, the Framingham Heart Study; GRS, genetic risk score; GWAS, genome-wide association studies; HDL-C, high density lipoprotein cholesterol; LDL-C, low density lipoprotein cholesterol; log-leptin, logarithmically transformed leptin; log-TG, logarithmically transformed TG; TC, total cholesterol; TG, triglycerides

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

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

Keywords: leptin, lipids, alcohol consumption, genetic risk score

Strengths and limitations of this study:

- 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 compared with observational epidemiology studies.
- The stringent quality control methods were used in measuring genotypes, phenotype, and covariates in the current study to reduce measurement error and increase the statistical power.
- Pleiotropy effects of SNPs included in the leptin genetic risk score (GRS) may confound the leptin GRS and lipids associations.
- Our analyses were restricted to individuals of European ancestry.

Introduction

Leptin is a key hormone that regulates appetite and food intake, body weight, and energy balance (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

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

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

Materials and Methods

Data Sources and Study Participants

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

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

Genotyping and Genetic Risk Score

Genetic loci for circulating leptin levels have been reported in a large genome-wide association studies (GWAS) meta-analysis by Kilpelainen and colleagues(Kilpelainen et al., 2016). This study included 32,161 individuals of European ancestry and identified three single-nucleotide polymorphisms (SNPs), *GCKR* rs780093, *LEP* rs10487505, and *SLC32A1* rs6071166, that were robustly associated with body mass index (BMI) adjusted leptin at a genome-wide significance level ($p<5\times10^{-08}$). In addition, *GCKR* rs780093, *CCNL1* rs900400, and *FTO* rs8043757 were associated with circulating leptin without adjustment for BMI(Kilpelainen et al., 2016). We assumed the additive genetic model for each SNP and constructed two genetic risk scores (GRSs) for leptin by combining leptin-increasing alleles for SNPs weighted by their corresponding effect sizes on logarithmically transformed leptin (log-leptin) as reported in the original GWAS meta-analysis(Kilpelainen et al., 2016). The first score, GRS1, was generated using the three SNPs associated with BMI adjusted leptin, and the second score, GRS2, using the three SNPs associated with leptin unadjusted for BMI.

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

removed SNPs with a Hardy-Weinberg equilibrium $p<1\times10^{-6}$, a missing rate>3.1%, a minor allele frequency (MAF)<1%, a missing physical position or cannot mapped to build 37 positions, Mendelian errors > 1000, or duplicate SNPs. MACH software was used for genotype phasing, followed by imputation using MiniMac software (Auton et al., 2015, Das et al., 2016). Imputation results were summarized as dosage scores, which represent the expected numbers of copies of the coded allele for each SNP, ranging from 0 to 2. After imputation, SNPs with $r^2<0.30$, an MAF<1%, or a Hardy-Weinberg equilibrium $p<1\times10^{-6}$ were removed. We retrieved genotypes of the SNPs for GRSs from the imputed data for all study participants (**Supplemental Table S1 and Supplemental Table S2**).

Leptin and Lipids measurement

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

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

Covariates

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

Statistical Analysis

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

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

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

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

Results

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

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

BMI independent leptin GRS1 and blood lipids

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

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

BMI dependent leptin GRS2 and blood lipids

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

When restricting to participants not taking lipid-lowering medication and those not taking lipid- or glucose-lowering medications, respectively, the associations of GRS1 and GRS2 with blood lipids were similar to those as shown above (**Supplemental Table S3, S4, S5** and S**6**).

Discussion

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

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

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

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

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

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

GRS and lipids associations. It is possible that our results may represent a shared genetic basis between leptin and lipids rather than a causal relationship. Second, we may not have sufficient power to detect associations between genetically determined leptin levels and LDL-C, HDL-C, and TC. Larger Mendelian randomization studies are warranted to evaluate associations between leptin and LDL-C, HDL-C, and TC. Finally, our analyses were restricted to individuals of European ancestry. Our findings may not be generalizable to populations of other ancestries.

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

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Author Contributions

Conceptualization, Changwei Li, José Cordero, Jia-Sheng Wang, and Shengxu Li; Formal analysis, Luqi Shen and Ye Shen; Supervision, Changwei Li, José Cordero, Jia-Sheng Wang, and Shengxu Li; Writing – original draft, Luqi Shen; Writing – review & editing, Changwei Li, José Cordero, and Luqi Shen.

Conflict of interest

Conflicts of interest and disclosures: none.

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References

1. (2010) 1000 Genome imputation based on the November 2010 release 1000 genome imputation general procedure, in Series 1000 Genome imputation based on the November 2010 release 1000 genome imputation general procedure.

2. Andersson C, Lyass A, Larson MG, Robins SJ, Vasan RS (2015) Low-density-lipoprotein cholesterol concentrations and risk of incident diabetes: epidemiological and genetic insights from the Framingham Heart Study. Diabetologia 58:2774-2780.

3. Auton A, Brooks LD, Durbin RM, Garrison EP, Kang HM, Korbel JO, Marchini JL, McCarthy S, McVean GA, Abecasis GR, Consortium GP (2015) A global reference for human genetic variation. Nature 526:68-74.

4. Ayina CN, Noubiap JJ, Etoundi Ngoa LS, Boudou P, Gautier JF, Mengnjo MK, Mbanya JC, Sobngwi E (2016) Association of serum leptin and adiponectin with anthropomorphic indices of obesity, blood lipids and insulin resistance in a Sub-Saharan African population. Lipids in health and disease 15:96.

5. Balasubramaniyan V, Nalini N (2006) Intraperitoneal leptin regulates lipid metabolism in ethanol supplemented Mus musculas heart. Life sciences 78:831-837.

6. Beulens JW, de Zoete EC, Kok FJ, Schaafsma G, Hendriks HF (2008) Effect of moderate alcohol consumption on adipokines and insulin sensitivity in lean and overweight men: a diet intervention study. European journal of clinical nutrition 62:1098-1105.

7. Campfield LA, Smith FJ, Guisez Y, Devos R, Burn P (1995) Recombinant mouse OB protein: evidence for a peripheral signal linking adiposity and central neural networks. Science 269:546-549.

8. Das S, Forer L, Schönherr S, Sidore C, Locke AE, Kwong A, Vrieze SI, Chew EY, Levy S, McGue M, Schlessinger D, Stambolian D, Loh PR, Iacono WG, Swaroop A, Scott LJ, Cucca F,

Kronenberg F, Boehnke M, Abecasis GR, Fuchsberger C (2016) Next-generation genotype imputation service and methods. Nat Genet 48:1284-1287.

9. Ebihara K, Kusakabe T, Hirata M, Masuzaki H, Miyanaga F, Kobayashi N, Tanaka T, Chusho H, Miyazawa T, Hayashi T, Hosoda K, Ogawa Y, DePaoli AM, Fukushima M, Nakao K (2007) Efficacy and safety of leptin-replacement therapy and possible mechanisms of leptin actions in patients with generalized lipodystrophy. The Journal of clinical endocrinology and metabolism 92:532-541.

10. Enser M, Ashwell M (1983) Fatty acid composition of triglycerides from adipose tissue transplanted between obese and lean mice. Lipids 18:776-780.

11. Friedewald WT, Levy RI, Fredrickson DS (1972) Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. Clinical chemistry 18:499-502.

12. Gao L, Zhou Y, Zhong W, Zhao X, Chen C, Chen X, Gu Y, Chen J, Lv Z, Shen J (2014) Caveolin-1 is essential for protecting against binge drinking-induced liver damage through inhibiting reactive nitrogen species. Hepatology 60:687-699.

13. Greco AV, Mingrone G, Favuzzi A, Capristo E, Gniuli D, Addolorato G, Brunani A, Cavagnin F, Gasbarrini G (2000) Serum leptin levels in post-hepatitis liver cirrhosis. Journal of hepatology 33:38-42.

14. Halaas JL, Gajiwala KS, Maffei M, Cohen SL, Chait BT, Rabinowitz D, Lallone RL, Burley SK, Friedman JM (1995) Weight-reducing effects of the plasma protein encoded by the obese gene. Science 269:543-546.

15. Harris RB (2014) Direct and indirect effects of leptin on adipocyte metabolism. Biochimica et biophysica acta 1842:414-423.

16. Henriksen JH, Holst JJ, Moller S, Brinch K, Bendtsen F (1999) Increased circulating leptin in alcoholic cirrhosis: relation to release and disposal. Hepatology 29:1818-1824.

17. Hynes GR, Jones PJ (2001) Leptin and its role in lipid metabolism. Curr Opin Lipidol 12:321-327.

18. Jaleel F, Jaleel A, Rahman MA, Alam E (2006) Comparison of adiponectin, leptin and blood lipid levels in normal and obese postmenopausal women. J Pak Med Assoc 56:391-394.

19. Javor ED, Cochran EK, Musso C, Young JR, Depaoli AM, Gorden P (2005) Long-term efficacy of leptin replacement in patients with generalized lipodystrophy. Diabetes 54:1994-2002.

20. Jürimäe T, Jürimäe J, Leppik A, Tafeit E, Kums T (2010) Relationships between adiponectin, leptin, and blood lipids in physically active postmenopausal females. Am J Hum Biol 22:609-612.

21. Kamran F, Rother KI, Cochran E, Safar Zadeh E, Gorden P, Brown RJ (2012) Consequences of stopping and restarting leptin in an adolescent with lipodystrophy. Hormone research in paediatrics 78:320-325.

22. Kannel WB, Sorlie P (1979) Some health benefits of physical activity. The Framingham Study. Archives of internal medicine 139:857-861.

23. Kavazarakis E, Moustaki M, Gourgiotis D, Drakatos A, Bossios A, Zeis PM, Xatzidimoula A, Karpathios T (2001) Relation of serum leptin levels to lipid profile in healthy children. Metabolism 50:1091-1094.

24. Kilpelainen TO, Carli JF, Skowronski AA, Sun Q, Kriebel J, Feitosa MF, Hedman AK, Drong AW, Hayes JE, Zhao J, Pers TH, Schick U, Grarup N, Kutalik Z, Trompet S, Mangino M, Kristiansson K, Beekman M, Lyytikainen LP, Eriksson J, Henneman P, Lahti J, Tanaka T, Luan

J, Del Greco MF, Pasko D, Renstrom F, Willems SM, Mahajan A, Rose LM, Guo X, Liu Y, Kleber ME, Perusse L, Gaunt T, Ahluwalia TS, Ju Sung Y, Ramos YF, Amin N, Amuzu A, Barroso I, Bellis C, Blangero J, Buckley BM, Bohringer S, YD IC, de Craen AJ, Crosslin DR, Dale CE, Dastani Z, Day FR, Deelen J, Delgado GE, Demirkan A, Finucane FM, Ford I, Garcia ME, Gieger C, Gustafsson S, Hallmans G, Hankinson SE, Havulinna AS, Herder C, Hernandez D, Hicks AA, Hunter DJ, Illig T, Ingelsson E, Ioan-Facsinay A, Jansson JO, Jenny NS, Jorgensen ME, Jorgensen T, Karlsson M, Koenig W, Kraft P, Kwekkeboom J, Laatikainen T, Ladwig KH, LeDuc CA, Lowe G, Lu Y, Marques-Vidal P, Meisinger C, Menni C, Morris AP, Myers RH, Mannisto S, Nalls MA, Paternoster L, Peters A, Pradhan AD, Rankinen T, Rasmussen-Torvik LJ, Rathmann W, Rice TK, Brent Richards J, Ridker PM, Sattar N, Savage DB, Soderberg S, Timpson NJ, Vandenput L, van Heemst D, Uh HW, Vohl MC, Walker M, Wichmann HE, Widen E, Wood AR, Yao J, Zeller T, Zhang Y, Meulenbelt I, Kloppenburg M, Astrup A, Sorensen TI, Sarzynski MA, Rao DC, Jousilahti P, Vartiainen E, Hofman A, Rivadeneira F, Uitterlinden AG, Kajantie E, Osmond C, Palotie A, Eriksson JG, Heliovaara M, Knekt PB, Koskinen S, Jula A, Perola M, Huupponen RK, Viikari JS, Kahonen M, Lehtimaki T, Raitakari OT, Mellstrom D, Lorentzon M, Casas JP, Bandinelli S, Marz W, Isaacs A, van Dijk KW, van Duijn CM, Harris TB, Bouchard C, Allison MA, Chasman DI, Ohlsson C, Lind L, Scott RA, Langenberg C, Wareham NJ, Ferrucci L, Frayling TM, Pramstaller PP, Borecki IB, Waterworth DM, Bergmann S, Waeber G, Vollenweider P, Vestergaard H, Hansen T, Pedersen O, Hu FB, Eline Slagboom P, Grallert H, Spector TD, Jukema JW, Klein RJ, Schadt EE, Franks PW, Lindgren CM, Leibel RL, Loos RJ (2016) Genome-wide meta-analysis uncovers novel loci influencing circulating leptin levels. Nature communications 7:10494.

25. Kosztaczky B, Foris G, Paragh G, Jr., Seres I, Zsiros E, Koncsos P, Balogh Z, Paragh G (2007) Leptin stimulates endogenous cholesterol synthesis in human monocytes: New role of an old player in atherosclerotic plaque formation. Leptin-induced increase in cholesterol synthesis. The international journal of biochemistry & cell biology 39:1637-1645.

26. Maddalozzo GF, Turner RT, Edwards CH, Howe KS, Widrick JJ, Rosen CJ, Iwaniec UT (2009) Alcohol alters whole body composition, inhibits bone formation, and increases bone marrow adiposity in rats. Osteoporosis international : a journal established as result of cooperation between the European Foundation for Osteoporosis and the National Osteoporosis Foundation of the USA 20:1529-1538.

27. Nicolas JM, Fernandez-Sola J, Fatjo F, Casamitjana R, Bataller R, Sacanella E, Tobias E, Badia E, Estruch R (2001) Increased circulating leptin levels in chronic alcoholism. Alcoholism, clinical and experimental research 25:83-88.

28. Otaka M, Konishi N, Odashima M, Jin M, Wada I, Matsuhashi T, Ohba R, Watanabe S (2007) Effect of alcohol consumption on leptin level in serum, adipose tissue, and gastric mucosa. Digestive diseases and sciences 52:3066-3069.

29. Palmer TM, Sterne JA, Harbord RM, Lawlor DA, Sheehan NA, Meng S, Granell R, Smith GD, Didelez V (2011) Instrumental variable estimation of causal risk ratios and causal odds ratios in Mendelian randomization analyses. Am J Epidemiol 173:1392-1403.

30. Park JY, Javor ED, Cochran EK, DePaoli AM, Gorden P (2007) Long-term efficacy of leptin replacement in patients with Dunnigan-type familial partial lipodystrophy. Metabolism: clinical and experimental 56:508-516.

31. Paz-Filho G, Mastronardi CA, Licinio J (2015) Leptin treatment: facts and expectations. Metabolism: clinical and experimental 64:146-156.

32. Pravdova E, Macho L, Fickova M (2009) Alcohol intake modifies leptin, adiponectin and resistin serum levels and their mRNA expressions in adipose tissue of rats. Endocrine regulations 43:117-125.

33. Rao DC, Sung YJ, Winkler TW, Schwander K, Borecki I, Cupples LA, Gauderman WJ, Rice K, Munroe PB, Psaty BM, Group* CG-LIW (2017) Multiancestry Study of Gene-Lifestyle Interactions for Cardiovascular Traits in 610 475 Individuals From 124 Cohorts: Design and Rationale. Circ Cardiovasc Genet 10.

34. Rojdmark S, Calissendorff J, Brismar K (2001) Alcohol ingestion decreases both diurnal and nocturnal secretion of leptin in healthy individuals. Clinical endocrinology 55:639-647.

35. Roth MJ, Baer DJ, Albert PS, Castonguay TW, Dorgan JF, Dawsey SM, Brown ED, Hartman TJ, Campbell WS, Giffen CA, Judd JT, Taylor PR (2003) Relationship between serum leptin levels and alcohol consumption in a controlled feeding and alcohol ingestion study. Journal of the National Cancer Institute 95:1722-1725.

36. Sainz N, Barrenetxe J, Moreno-Aliaga MJ, Martinez JA (2015) Leptin resistance and diet-induced obesity: central and peripheral actions of leptin. Metabolism: clinical and experimental 64:35-46.

37. Santolaria F, Perez-Cejas A, Aleman MR, Gonzalez-Reimers E, Milena A, de la Vega MJ, Martinez-Riera A, Gomez-Rodriguez MA (2003) Low serum leptin levels and malnutrition in chronic alcohol misusers hospitalized by somatic complications. Alcohol and alcoholism 38:60-66.

38. Sekhar RV, Jahoor F, Iyer D, Guthikonda A, Paranilam J, Elhaj F, Coraza I, Balasubramanyam A (2012) Leptin replacement therapy does not improve the abnormal lipid

kinetics of hypoleptinemic patients with HIV-associated lipodystrophy syndrome. Metabolism 61:1395-1403.

39. Selenscig D, Rossi A, Chicco A, Lombardo YB (2010) Increased leptin storage with altered leptin secretion from adipocytes of rats with sucrose-induced dyslipidemia and insulin resistance: effect of dietary fish oil. Metabolism: clinical and experimental 59:787-795.

40. Singh P, Peterson TE, Sert-Kuniyoshi FH, Glenn JA, Davison DE, Romero-Corral A, Pusalavidyasagar S, Jensen MD, Somers VK (2012) Leptin signaling in adipose tissue: role in lipid accumulation and weight gain. Circ Res 111:599-603.

41. Slomiany BL, Slomiany A (2009) Role of epidermal growth factor receptor transactivation in the activation of cytosolic phospholipase A(2) in leptin protection of salivary gland acinar cells against ethanol cytotoxicity. Journal of physiology and pharmacology : an official journal of the Polish Physiological Society 60:49-55.

42. Splansky GL, Corey D, Yang Q, Atwood LD, Cupples LA, Benjamin EJ, D'Agostino RB, Sr., Fox CS, Larson MG, Murabito JM, O'Donnell CJ, Vasan RS, Wolf PA, Levy D (2007) The Third Generation Cohort of the National Heart, Lung, and Blood Institute's Framingham Heart Study: design, recruitment, and initial examination. American journal of epidemiology 165:1328-1335.

43. Tan X, Sun X, Li Q, Zhao Y, Zhong W, Sun X, Jia W, McClain CJ, Zhou Z (2012) Leptin deficiency contributes to the pathogenesis of alcoholic fatty liver disease in mice. The American journal of pathology 181:1279-1286.

44. Voican CS, Njike-Nakseu M, Boujedidi H, Barri-Ova N, Bouchet-Delbos L, Agostini H, Maitre S, Prevot S, Cassard-Doulcier AM, Naveau S, Perlemuter G (2015) Alcohol withdrawal

alleviates adipose tissue inflammation in patients with alcoholic liver disease. Liver international : official journal of the International Association for the Study of the Liver 35:967-978.

45. Wang MY, Lee Y, Unger RH (1999) Novel form of lipolysis induced by leptin. The Journal of biological chemistry 274:17541-17544.

46. Wannamethee SG, Tchernova J, Whincup P, Lowe GD, Kelly A, Rumley A, Wallace AM, Sattar N (2007) Plasma leptin: associations with metabolic, inflammatory and haemostatic risk factors for cardiovascular disease. Atherosclerosis 191:418-426.

47. Wu DM, Shen MH, Chu NF (2001) Relationship between plasma leptin levels and lipid profiles among school children in Taiwan--the Taipei Children Heart Study. European journal of epidemiology 17:911-916.

48. Yu HC, Li SY, Cao MF, Jiang XY, Feng L, Zhao JJ, Gao L (2010) Effects of chronic ethanol consumption on levels of adipokines in visceral adipose tissues and sera of rats. Acta pharmacologica Sinica 31:461-469.

49. Zhang Y, Proenca R, Maffei M, Barone M, Leopold L, Friedman JM (1994) Positional cloning of the mouse obese gene and its human homologue. Nature 372:425-432.

Figure legends

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

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 (n=3,860)	Quartiles of the leptin GRS				
		Q1 (n=964)	Q2 (n=961)	Q3 (n=977)	Q4 (n=958)	*P*
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 (%)						
No more than high school	591 (15.4)	141 (14.7)	146 (15.3)	157 (16.1)	147 (15.4)	
Some college	1213 (31.5)	306 (31.8)	287 (30.0)	313 (32.1)	307 (32.3)	0.22
Bachelor's degree and above	2041 (53.1)	514 (53.5)	524 (54.8)	505 (51.8)	498 (52.3)	
Current Smoker, N (%)	603 (15.6)	144 (15.0)	152 (15.8)	165 (16.9)	142 (14.8)	0.53
Current Drinker, N (%)	3419 (89.1)	858 (89.4)	853 (89.1)	863 (88.9)	845 (89.0)	0.32
Physical Activities index score, mean(SD)	37.5 (7.9)	37.8 (8.0)	37.3 (8.1)	37.4 (7.7)	37.4 (7.8)	0.51
BMI, kg/m2, 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		$P_{interaction}$ [b]	Fully adjusted model[c]		$P_{interaction}$ [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 GRS2[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		$P_{interaction}$ [b]	Fully adjusted model[c]		$P_{interaction}$ [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.

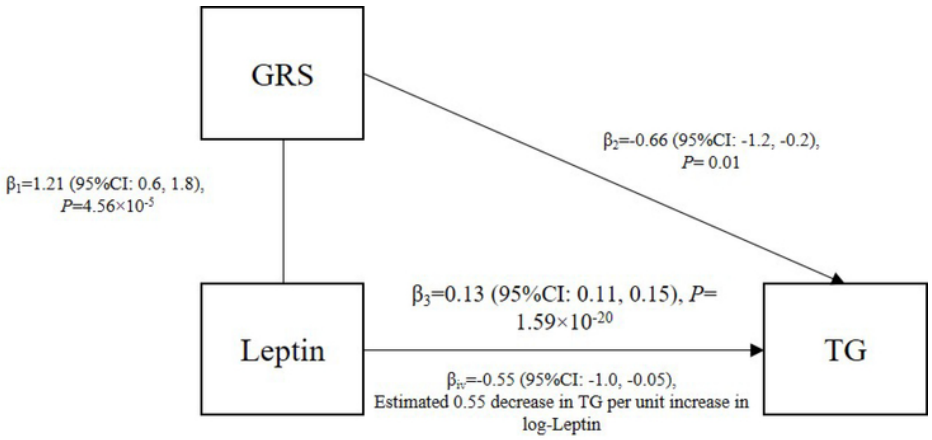

Figure 1

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Supplemental Table S1. Basic Information of three SNPs for BMI independent leptin GRS1[a] reaching genome-wide significance ($P<5\times10^{-8}$)

Chromosome Position	rsID	Coded Allele	Non-coded Allele	R^2	Function	Nearest 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 ($P<5\times10^{-8}$)

Chromosome Position	rsID	Coded Allele	Non-coded Allele	R^2	Function	Nearest 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		$P_{interaction}$ [b]	Fully adjusted model[c]		$P_{interaction}$ [d]
	Beta(SE)	*P*		Beta(SE)	*P*	
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		$P_{interaction}$[b]	Fully adjusted model[c]		$P_{interaction}$[d]
	Beta(SE)	*P*		Beta(SE)	*P*	
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		$P_{interaction}$ [b]	Fully adjusted model[c]		$P_{interaction}$ [d]
	Beta(SE)	*P*		Beta(SE)	*P*	
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.

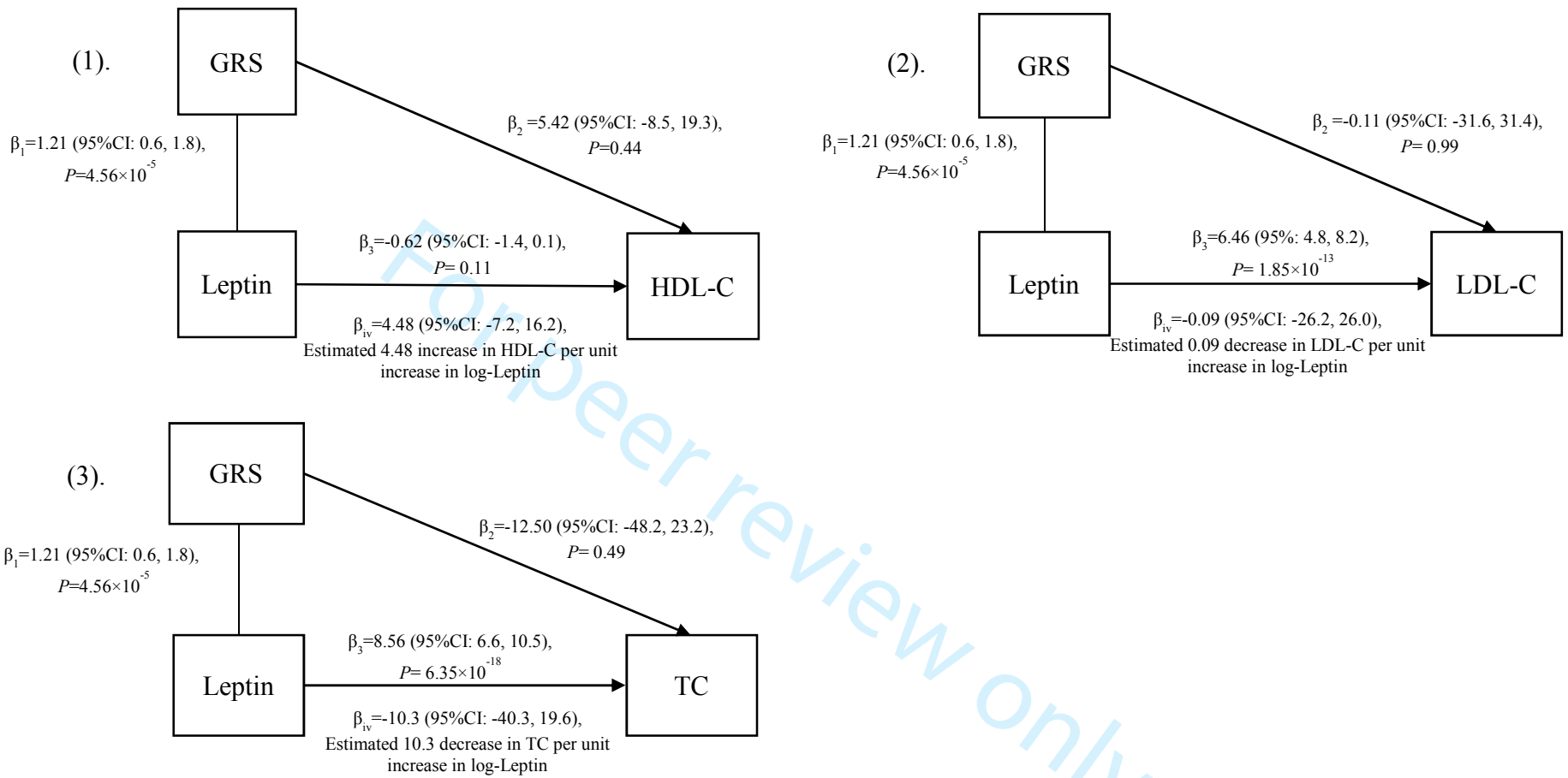
Supplemental Table S6. Association of BMI dependent Leptin GRS2[a] with Baseline Lipids among Overall, Drinking, and Non-Drinking Participants without treating for Lipids and Diabetes of the Framingham Heart Study 3rd Generation Cohort, respectively.

	Age, sex, BMI, waist adjusted model		$P_{interaction}$ [b]	Fully adjusted model[c]		$P_{interaction}$ [d]
	Beta(SE)	*P*		Beta(SE)	*P*	
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	
			0.06			0.08
Not current drinkers	-2.00 (0.75)	0.008		-1.87 (0.76)	0.01	

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	

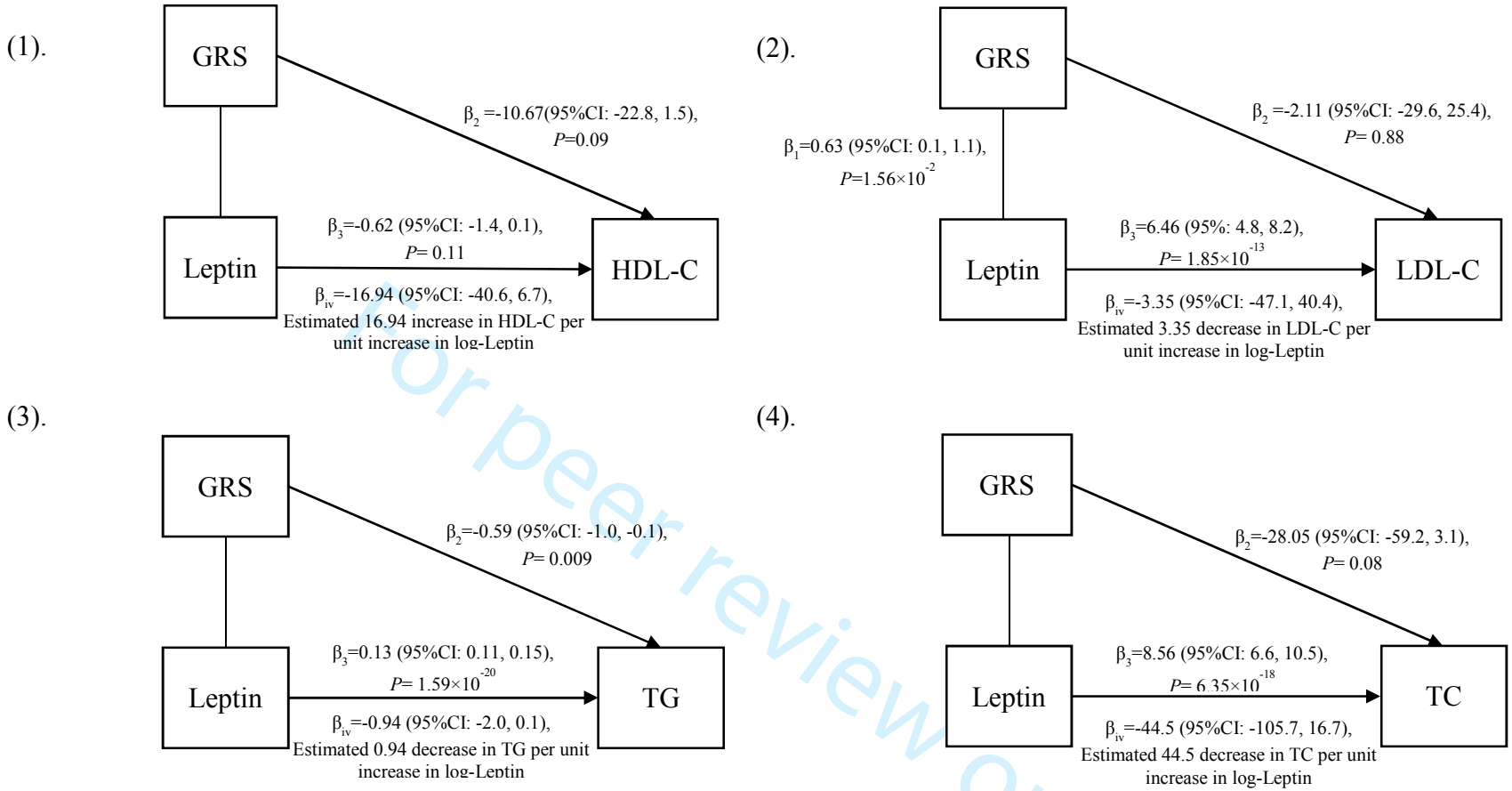
BMI=body mass index; GRS=Genetic Risk Score; HDL-C=High-density lipoprotein cholesterol; LDL-C=Low-density lipoprotein cholesterol; log-TG=logarithmically transformed triglycerides; SE=standard error

[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)

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

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.

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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

#6a	Cohort study – Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up. Case-control study – Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls. Cross-sectional study – Give the eligibility criteria, and the sources and methods of selection of participants. Give information on the criteria and methods for selection of subsets of participants from a larger study, when relevant.	5-6
#6b	Cohort study – For matched studies, give matching criteria and number of exposed and unexposed. Case-control study – For matched studies, give matching criteria and the number of controls per case.	n/a
#7a	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	7-8
#7b	Clearly define genetic exposures (genetic variants) using a widely-used nomenclature system. Identify variables likely to be associated with population stratification (confounding by ethnic origin).	6-7
#8a	For each variable of interest give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group. Give information separately for for exposed and unexposed groups if applicable.	5-6
#8b	Describe laboratory methods, including source and storage of DNA, genotyping methods and platforms (including the allele calling algorithm used, and its version), error rates and call rates. State the laboratory / centre where genotyping was done. Describe comparability of laboratory methods if there is more than one group. Specify whether genotypes were assigned using all of the data from the study simultaneously or in smaller batches.	6-7
#9a	Describe any efforts to address potential sources of bias	8-9
#9b	Describe any efforts to address potential sources of bias	8-9
#10	Explain how the study size was arrived at	5-6
#11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen, and why. If applicable, describe how effects of treatment were dealt with.	6
#12a	Describe all statistical methods, including those used to control for	8-9

	confounding. State software version used and options (or settings) chosen.	
#12b	Describe any methods used to examine subgroups and interactions	9
#12c	Explain how missing data were addressed	9
#12d	If applicable, explain how loss to follow-up was addressed	n/a
#12e	Describe any sensitivity analyses	9
#12f	State whether Hardy-Weinberg equilibrium was considered and, if so, how.	7
#12g	Describe any methods used for inferring genotypes or haplotypes	7
#12h	Describe any methods used to assess or address population stratification.	7
#12i	Describe any methods used to address multiple comparisons or to control risk of false positive findings.	7
#12j	Describe any methods used to address and correct for relatedness among subjects	n/a
#13a	Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed. Give information separately for for exposed and unexposed groups if applicable. Report numbers of individuals in whom genotyping was attempted and numbers of individuals in whom genotyping was successful.	5-6
#13b	Give reasons for non-participation at each stage	n/a
#13c	Consider use of a flow diagram	n/a
#14a	Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders. Give information separately for exposed and unexposed groups if applicable. Consider giving information by genotype	9-10
#14b	Indicate number of participants with missing data for each variable of interest	9-10
#14c	Cohort study – Summarize follow-up time, e.g. average and total amount.	n/a

#15	Cohort study Report numbers of outcome events or summary measures over time.Give information separately for exposed and unexposed groups if applicable. Report outcomes (phenotypes) for each genotype category over time Case-control study – Report numbers in each exposure category, or summary measures of exposure.Give information separately for cases and controls . Report numbers in each genotype category. Cross-sectional study – Report numbers of outcome events or summary measures. Give information separately for exposed and unexposed groups if applicable. Report outcomes (phenotypes) for each genotype category	9/10
#16a	Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	10-11
#16b	Report category boundaries when continuous variables were categorized	10-11
#16c	If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	10-11
#16d	Report results of any adjustments for multiple comparisons	10-11
#17a	Report other analyses done—e.g., analyses of subgroups and interactions, and sensitivity analyses	10-11
#17b	Report other analyses done—e.g., analyses of subgroups and interactions, and sensitivity analyses	10-11
#17c	Report other analyses done—e.g., analyses of subgroups and interactions, and sensitivity analyses	10-11
#18	Summarise key results with reference to study objectives	11-13
#19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias.	13-14
#20	Give a cautious overall interpretation considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence.	14
#21	Discuss the generalisability (external validity) of the study results	14
#22	Give the source of funding and the role of the funders for the present	15

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

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

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

Running title: Genetically determined leptin and lipids

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Abstract

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

Design: We conducted a Mendelian 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) (β=-0.66, p =0.01), but not low density lipoprotein cholesterol (LDL-C) (p=0.99), high density lipoprotein cholesterol (HDL-C) (p=0.44), or total cholesterol (TC) (p=0.49). Instrumental variable estimation showed that per unit increase in genetically determined log-leptin was associated with 0.55 (95% confidence interval: 0.05-1.00) units decrease in log-TG. Besides significant association with log-TG (β=-0.59, p=0.009), the BMI dependent GRS was nominally associated with HDL-C (β=-10.67, p=0.09) and TC (β=-28.05, p=0.08). When stratified by drinking status, the BMI dependent GRS was associated with reduced levels of LDL-C (p=0.03), log-TG (p=0.004), and TC (p=0.003) among non-current drinkers only. Significant interactions between the BMI dependent GRS and alcohol drinking were identified for LDL-C (p=0.03), 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.

Keywords: leptin, lipids, alcohol consumption, genetic risk score

Strengths and limitations of this study:

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

Introduction

Leptin is a key hormone that regulates appetite and food intake, body weight, and energy balance.[1 2] Leptin is secreted primarily from the stomach, placenta, and adipose tissue.[3] Biological studies have demonstrated that elevated leptin levels may play an important role in the pathogenesis of lipid accumulation.[4-9] As an 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-cells[13] and improves insulin sensitivity[14]. 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

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.[27-40] In rodent models, leptin has been demonstrated to be increased [30-32] or decreased [33 34] after alcohol intake. Similarly, leptin levels in human was decreased,[36] increased,[35 37] or even unchanged [38-40] after drinking. It is unclear whether alcohol consumption modifies the association of genetically determined leptin with lipid levels.[41 42]

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

Materials and Methods

Data Sources and Study Participants

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

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

Genotyping and Genetic Risk Score

Genetic loci for circulating leptin levels have been reported in a large genome-wide association studies (GWAS) meta-analysis by Kilpelainen and colleagues.[26] This study included 32,161 individuals of European ancestry and identified three single-nucleotide polymorphisms (SNPs), *GCKR* rs780093, *LEP* rs10487505, and *SLC32A1* rs6071166, that were robustly associated with body mass index (BMI) adjusted leptin at a genome-wide significance level ($p<5\times10^{-08}$). In addition, *GCKR* rs780093, *CCNL1* rs900400, and *FTO* rs8043757 were associated with circulating leptin without adjustment for BMI.[26] We assumed the additive genetic model for each SNP and constructed two genetic risk scores (GRSs) for leptin by combining leptin-increasing alleles for SNPs weighted by their corresponding effect sizes on logarithmically transformed leptin (log-leptin) as reported in the original GWAS meta-analysis.[26] The first score, GRS1, was generated using the three SNPs associated with BMI adjusted leptin, and the second score, GRS2, using the three SNPs associated with leptin unadjusted for BMI.

Genome-wide SNPs were genotyped using Affymetrix and Illumina platforms in the FHS. The 1000 Genome genotype data for the FHS was already imputed and cataloged on the dbGaP. According to the document of the FHS,[44] before imputation, quality control removed SNPs with a Hardy-Weinberg equilibrium $p<1\times10^{-6}$, a missing rate>3.1%, a minor allele frequency (MAF)<1%, a missing physical position or cannot mapped to build 37 positions, Mendelian errors > 1000, or duplicate SNPs. MACH software was used for genotype phasing, followed by imputation using MiniMac software.[45 46] Imputation results were summarized as

dosage scores, which represent the expected numbers of copies of the coded allele for each SNP, ranging from 0 to 2. After imputation, SNPs with r^2<0.30, an MAF<1%, or a Hardy-Weinberg equilibrium $p<1\times10^{-6}$ were removed. We retrieved genotypes of the SNPs for GRSs from the imputed data for all study participants (**Supplemental Table S1 and Supplemental Table S2**).

Leptin and Lipids measurement

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

Fasting blood lipids, including TC, HDL-C, and TG, were measured using automated enzymatic assays.[47] For participants taking lipid-lowering medications, TC was adjusted as TC/0.8.[48] After adjustment, LDL-C was calculated using the Friedewald formula.[49] The adjusted TC and LDL-C and logarithmically transformed TG (log-TG) were used for analyses in the current study.

Covariates

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

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

Statistical Analysis

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

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

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

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

Participant and Public Involvement

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

Results

Characteristics of the study participants are presented in **Table 1**. Participants were on average 40.2 years old at baseline. There were slightly more females (53.2%), and only 15.4% had less than a high school education. The majority (89.1%) of the participants were current drinkers, and 15.6% were current smokers. Participants were on average over weighted, with a mean BMI of 26.9 kg/m^2 and mean waist girth of 36.6 inches. About 6.9% of the participants

were treated for dyslipidemia, and 1.9% were treated for diabetes. The BMI independent leptin GRS1 was not associated with age (p=0.23), sex (p=0.89), education (p=0.22), smoking (p=0.53), drinking (p=0.32), BMI (p=0.94), waist circumference (p=0.70), lipid-lowering medication usage (p=0.26), or the physical activity index score (p=0.51), but with diabetes-lowering medication usage (p=0.03). As expected, the GRS1 was positively associated with age, sex, BMI, and waist circumference adjusted log-leptin ($p=4.56\times10^{-5}$).

BMI independent leptin GRS1 and blood lipids

After controlling for age, sex, BMI, and waist circumference, log-leptin was positively associated with TC (β=8.56, $p=6.35\times10^{-18}$), LDL-C (β=6.46, $p=1.85\times10^{-13}$), and log-TG (β=0.13, $p=1.59\times10^{-20}$), but was not associated with HDL-C (β=-0.62, p=0.11) (**Figure 1 and Supplemental Figure S1**). Per unit increase in the leptin GRS1 was associated with a 1.21-unit increase in the age, sex, BMI, and waist circumference adjusted log-leptin ($p=4.56\times10^{-5}$). The leptin GRS1 was inversely associated with age, sex, BMI, and waist circumference adjusted log-TG (β=-0.66, p=0.01) (**Figure 1**). When further adjusting for education, smoking, drinking, and physical activity, the GRS1 and log-TG association was still significant (β=-0.69, p=0.008, **Table 2**). Instrumental variable estimation indicated that log-TG levels decreased by 0.55 (95% CI: 0.05, 1.00, p=0.02) per unit increase of genetically determined log-leptin level (**Figure 1**). The leptin GRS1 was inversely associated with TC (β=-12.50, p=0.49) and LDL-C (β=-0.11, p=0.99) and positively associated with HDL-C (β=5.42, p=0.44), however, the correlations were not significant. The GRS1 and blood lipids associations were not modified by drinking status (**Table 2**).

BMI dependent leptin GRS2 and blood lipids

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

When restricting to participants not taking lipid-lowering medication and those not taking lipid- or glucose-lowering medications, respectively, the associations of GRS1 and GRS2 with blood lipids were similar to those as shown above (**Supplemental Table S3, S4, S5** and S**6**).

Discussion

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

Both the BMI dependent- and independent- GRSs were associated with lower level of log-TG in the current study. Inconsistent associations between leptin and blood lipids have been observed in previous studies. In a small study of 80 postmenopausal women, serum leptin was positively associated with HDL-C, TG, and TC, and inversely associated with LDL-C.[52] Another study conducted with 294 healthy school children reported that leptin was only associated with increased TG.[53] However, a study of 476 residents from Cameroon reported a positive correlation between leptin, LDL-C, and TC, and a positive association between leptin and TC, but no association between leptin and HDL-C or TG.[54] In a more recent study of 134 physically active postmenopausal women, no significant correlation was detected for leptin and blood lipids.[55] The divergent results of previous studies make it impossible to infer a relationship between leptin and blood lipids. Possible reasons for the divergent findings include varying sample sizes, failure to account for residual and unmeasured confounding, and the genetic background of the study population. Through Mendelian randomization analyses, we demonstrated that genetically determined leptin was inversely associated with log-TG. It is well known that alleles, such as risk alleles for leptin, are randomly assigned at meiosis and therefore, are independent of non-genetic confounders. The association between leptin GRS and log-TG in the current study was less prone to confounding. This also highlights the importance of using Mendelian randomization to delineate causal relationships. Our finding is further supported by previous physiologic studies, among which, leptin was demonstrated to inhibit lipogenesis, stimulate lipolysis, and reduce triglyceride uptake.[56] However, the association of HDL-C and TC were only nominally significant with BMI dependent GRS2 in the current study. It could be due to lack of statistical power or existing interaction of leptin and drinking. Therefore, we cannot rule out causal relationships between leptin and those lipid measures. Future large-scale

Mendelian randomization studies are warranted to evaluate associations of leptin GRS with HDL-C, LDL-C, and TC.

The BMI independent GRS1 was only associated with log-TG, while the BMI dependent GRS2 was also in nominal associations with HDL-C and TC. In addition, alcohol drinking modified the GRS2-lipids associations but not the GRS1-lipids associations. This indicated that the role of leptin in blood lipids regulation may be through multiple mechanisms. The BMI dependent GRS2 were inversely associated with LDL-C, log-TG, and TC only among non-current drinkers, but not among current drinkers. Although future studies are warranted to confirm these interactions, previous physiological studies may provide a reasonable explanation. Singh and colleagues demonstrated that the increased expression of caveolin-1 impairs leptin signaling and attenuates leptin-dependent effect to prevent lipid accumulation in human white pre-adipocytes.[57] Meanwhile, Caveolin-1 can be increased by alcohol drinking.[58]

Our study represents the first Mendelian randomization analyses for leptin and blood lipids in a population of European ancestry. A major strength of this study is the stringent quality control methods used in measuring genotypes, phenotype, and covariates in the FHS 3rd Generation Cohort. Those methods can reduce measurement error and increase the statistical power needed to identify associations between leptin GRS and lipids. We also identify some limitations. First, pleiotropy effects of SNPs included in the leptin GRS may confound the leptin GRS and lipids associations. It is possible that our results may represent a shared genetic basis between leptin and lipids rather than a causal relationship. Second, we may not have sufficient power to detect associations between genetically determined leptin levels and LDL-C, HDL-C, and TC. Larger Mendelian randomization studies are warranted to evaluate associations between

leptin and LDL-C, HDL-C, and TC. Third, we did not control for total energy intake in our analyses because food frequency questionnaire survey was not conducted in the third generation cohort at baseline when leptin was measured. However, leptin combines with receptors in the hypothalamus to reduce appetite and increase energy expenditure. Therefore, total energy intake is in the pathway from leptin to lipids metabolism and may not meet the criteria of being a confounder. Forth, the type of alcohol consumed was not measured and cannot be considered in the current analyses. It is possible that the alcohol consumed in the studied population is mainly wine and/or beers, which contain high level of resveratrol and phytochemical. The two chemicals may benefit lipid metabolism.[59 60] However, the two chemicals do not share similar genetic profile with leptin, and consequently, they should not be correlated with leptin and cannot affect the associations between leptin GRS and blood lipids. Finally, our analyses were restricted to individuals of European ancestry. Our findings may not be generalizable to populations of other ancestries.

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

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Author Contributions

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

Conflict of interest

Conflicts of interest and disclosures: none.

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Data sharing statement

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

References

1. Campfield LA, Smith FJ, Guisez Y, et al. Recombinant mouse OB protein: evidence for a peripheral signal linking adiposity and central neural networks. *Science* 1995;269(5223):546-9.
2. Halaas JL, Gajiwala KS, Maffei M, et al. Weight-reducing effects of the plasma protein encoded by the obese gene. *Science* 1995;269(5223):543-6.
3. Zhang Y, Proenca R, Maffei M, et al. Positional cloning of the mouse obese gene and its human homologue. *Nature* 1994;372(6505):425-32. doi: 10.1038/372425a0
4. Sainz N, Barrenetxe J, Moreno-Aliaga MJ, et al. Leptin resistance and diet-induced obesity: central and peripheral actions of leptin. *Metabolism: clinical and experimental* 2015;64(1):35-46. doi: 10.1016/j.metabol.2014.10.015
5. Wang MY, Lee Y, Unger RH. Novel form of lipolysis induced by leptin. *The Journal of biological chemistry* 1999;274(25):17541-4.
6. Harris RB. Direct and indirect effects of leptin on adipocyte metabolism. *Biochimica et biophysica acta* 2014;1842(3):414-23. doi: 10.1016/j.bbadis.2013.05.009
7. Enser M, Ashwell M. Fatty acid composition of triglycerides from adipose tissue transplanted between obese and lean mice. *Lipids* 1983;18(11):776-80.
8. Selenscig D, Rossi A, Chicco A, et al. Increased leptin storage with altered leptin secretion from adipocytes of rats with sucrose-induced dyslipidemia and insulin resistance: effect of dietary fish oil. *Metabolism: clinical and experimental* 2010;59(6):787-95. doi: 10.1016/j.metabol.2009.09.025
9. Kosztaczky B, Foris G, Paragh G, Jr., et al. Leptin stimulates endogenous cholesterol synthesis in human monocytes: New role of an old player in atherosclerotic plaque

formation. Leptin-induced increase in cholesterol synthesis. *The international journal of biochemistry & cell biology* 2007;39(9):1637-45. doi: 10.1016/j.biocel.2007.04.012

10. Francisco V, Pino J, Campos-Cabaleiro V, et al. Obesity, Fat Mass and Immune System: Role for Leptin. *Front Physiol* 2018;9:640. doi: 10.3389/fphys.2018.00640 [published Online First: 2018/06/01]

11. La Cava A. Leptin in inflammation and autoimmunity. *Cytokine* 2017;98:51-58. doi: 10.1016/j.cyto.2016.10.011

12. Maurya R, Bhattacharya P, Dey R, et al. Leptin Functions in Infectious Diseases. *Front Immunol* 2018;9:2741. doi: 10.3389/fimmu.2018.02741 [published Online First: 2018/11/26]

13. D'souza AM, Neumann UH, Glavas MM, et al. The glucoregulatory actions of leptin. *Mol Metab* 2017;6(9):1052-65. doi: 10.1016/j.molmet.2017.04.011 [published Online First: 2017/05/04]

14. Sivitz WI, Walsh SA, Morgan DA, et al. Effects of leptin on insulin sensitivity in normal rats. *Endocrinology* 1997;138(8):3395-401. doi: 10.1210/endo.138.8.5327

15. Barr VA, Malide D, Zarnowski MJ, et al. Insulin stimulates both leptin secretion and production by rat white adipose tissue. *Endocrinology* 1997;138(10):4463-72. doi: 10.1210/endo.138.10.5451

16. Saladin R, De Vos P, Guerre-Millo M, et al. Transient increase in obese gene expression after food intake or insulin administration. *Nature* 1995;377(6549):527-9. doi: 10.1038/377527a0

17. Abella V, Scotece M, Conde J, et al. Leptin in the interplay of inflammation, metabolism and immune system disorders. *Nat Rev Rheumatol* 2017;13(2):100-09. doi: 10.1038/nrrheum.2016.209 [published Online First: 2017/01/05]

18. Saltiel AR, Kahn CR. Insulin signalling and the regulation of glucose and lipid metabolism. *Nature* 2001;414(6865):799-806. doi: 10.1038/414799a

19. Paz-Filho G, Mastronardi CA, Licinio J. Leptin treatment: facts and expectations. *Metabolism: clinical and experimental* 2015;64(1):146-56. doi: 10.1016/j.metabol.2014.07.014

20. Park JY, Javor ED, Cochran EK, et al. Long-term efficacy of leptin replacement in patients with Dunnigan-type familial partial lipodystrophy. *Metabolism: clinical and experimental* 2007;56(4):508-16. doi: 10.1016/j.metabol.2006.11.010

21. Javor ED, Cochran EK, Musso C, et al. Long-term efficacy of leptin replacement in patients with generalized lipodystrophy. *Diabetes* 2005;54(7):1994-2002.

22. Ebihara K, Kusakabe T, Hirata M, et al. Efficacy and safety of leptin-replacement therapy and possible mechanisms of leptin actions in patients with generalized lipodystrophy. *The Journal of clinical endocrinology and metabolism* 2007;92(2):532-41. doi: 10.1210/jc.2006-1546

23. Kamran F, Rother KI, Cochran E, et al. Consequences of stopping and restarting leptin in an adolescent with lipodystrophy. *Hormone research in paediatrics* 2012;78(5-6):320-5. doi: 10.1159/000341398

24. Wu DM, Shen MH, Chu NF. Relationship between plasma leptin levels and lipid profiles among school children in Taiwan--the Taipei Children Heart Study. *European journal of epidemiology* 2001;17(10):911-6.

25. Sekhar RV, Jahoor F, Iyer D, et al. Leptin replacement therapy does not improve the abnormal lipid kinetics of hypoleptinemic patients with HIV-associated lipodystrophy syndrome. *Metabolism: clinical and experimental* 2012;61(10):1395-403. doi: 10.1016/j.metabol.2012.03.013

26. Kilpelainen TO, Carli JF, Skowronski AA, et al. Genome-wide meta-analysis uncovers novel loci influencing circulating leptin levels. *Nature communications* 2016;7:10494. doi: 10.1038/ncomms10494

27. Rojdmark S, Calissendorff J, Brismar K. Alcohol ingestion decreases both diurnal and nocturnal secretion of leptin in healthy individuals. *Clinical endocrinology* 2001;55(5):639-47.

28. Roth MJ, Baer DJ, Albert PS, et al. Relationship between serum leptin levels and alcohol consumption in a controlled feeding and alcohol ingestion study. *Journal of the National Cancer Institute* 2003;95(22):1722-5.

29. Otaka M, Konishi N, Odashima M, et al. Effect of alcohol consumption on leptin level in serum, adipose tissue, and gastric mucosa. *Digestive diseases and sciences* 2007;52(11):3066-9. doi: 10.1007/s10620-006-9635-x

30. Pravdova E, Macho L, Fickova M. Alcohol intake modifies leptin, adiponectin and resistin serum levels and their mRNA expressions in adipose tissue of rats. *Endocrine regulations* 2009;43(3):117-25.

31. Slomiany BL, Slomiany A. Role of epidermal growth factor receptor transactivation in the activation of cytosolic phospholipase A(2) in leptin protection of salivary gland acinar cells against ethanol cytotoxicity. *Journal of physiology and pharmacology : an official journal of the Polish Physiological Society* 2009;60(2):49-55.

32. Yu HC, Li SY, Cao MF, et al. Effects of chronic ethanol consumption on levels of adipokines in visceral adipose tissues and sera of rats. *Acta pharmacologica Sinica* 2010;31(4):461-9. doi: 10.1038/aps.2010.12

33. Maddalozzo GF, Turner RT, Edwards CH, et al. Alcohol alters whole body composition, inhibits bone formation, and increases bone marrow adiposity in rats. *Osteoporosis international : a journal established as result of cooperation between the European Foundation for Osteoporosis and the National Osteoporosis Foundation of the USA* 2009;20(9):1529-38. doi: 10.1007/s00198-009-0836-y

34. Tan X, Sun X, Li Q, et al. Leptin deficiency contributes to the pathogenesis of alcoholic fatty liver disease in mice. *The American journal of pathology* 2012;181(4):1279-86. doi: 10.1016/j.ajpath.2012.06.013

35. Henriksen JH, Holst JJ, Moller S, et al. Increased circulating leptin in alcoholic cirrhosis: relation to release and disposal. *Hepatology* 1999;29(6):1818-24. doi: 10.1002/hep.510290601

36. Santolaria F, Perez-Cejas A, Aleman MR, et al. Low serum leptin levels and malnutrition in chronic alcohol misusers hospitalized by somatic complications. *Alcohol and alcoholism* 2003;38(1):60-6.

37. Nicolas JM, Fernandez-Sola J, Fatjo F, et al. Increased circulating leptin levels in chronic alcoholism. *Alcoholism, clinical and experimental research* 2001;25(1):83-8.

38. Beulens JW, de Zoete EC, Kok FJ, et al. Effect of moderate alcohol consumption on adipokines and insulin sensitivity in lean and overweight men: a diet intervention study. *European journal of clinical nutrition* 2008;62(9):1098-105. doi: 10.1038/sj.ejcn.1602821

39. Voican CS, Njike-Nakseu M, Boujedidi H, et al. Alcohol withdrawal alleviates adipose tissue inflammation in patients with alcoholic liver disease. *Liver international : official journal of the International Association for the Study of the Liver* 2015;35(3):967-78. doi: 10.1111/liv.12575

40. Greco AV, Mingrone G, Favuzzi A, et al. Serum leptin levels in post-hepatitis liver cirrhosis. *Journal of hepatology* 2000;33(1):38-42.

41. Wannamethee SG, Tchernova J, Whincup P, et al. Plasma leptin: associations with metabolic, inflammatory and haemostatic risk factors for cardiovascular disease. *Atherosclerosis* 2007;191(2):418-26. doi: 10.1016/j.atherosclerosis.2006.04.012

42. Balasubramaniyan V, Nalini N. Intraperitoneal leptin regulates lipid metabolism in ethanol supplemented Mus musculas heart. *Life sciences* 2006;78(8):831-7. doi: 10.1016/j.lfs.2005.05.079

43. Splansky GL, Corey D, Yang Q, et al. The Third Generation Cohort of the National Heart, Lung, and Blood Institute's Framingham Heart Study: design, recruitment, and initial examination. *American journal of epidemiology* 2007;165(11):1328-35. doi: 10.1093/aje/kwm021

44. 1000 Genome imputation based on the November 2010 release 1000 genome imputation general procedure, 2010.

45. Auton A, Brooks LD, Durbin RM, et al. A global reference for human genetic variation. *Nature* 2015;526(7571):68-74. doi: 10.1038/nature15393

46. Das S, Forer L, Schönherr S, et al. Next-generation genotype imputation service and methods. *Nat Genet* 2016;48(10):1284-7. doi: 10.1038/ng.3656 [published Online First: 2016/08/29]

47. Andersson C, Lyass A, Larson MG, et al. Low-density-lipoprotein cholesterol concentrations and risk of incident diabetes: epidemiological and genetic insights from the Framingham Heart Study. *Diabetologia* 2015;58(12):2774-80. doi: 10.1007/s00125-015-3762-x

48. Rao DC, Sung YJ, Winkler TW, et al. Multiancestry Study of Gene-Lifestyle Interactions for Cardiovascular Traits in 610 475 Individuals From 124 Cohorts: Design and Rationale. *Circ Cardiovasc Genet* 2017;10(3) doi: 10.1161/CIRCGENETICS.116.001649

49. Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. *Clinical chemistry* 1972;18(6):499-502.

50. Kannel WB, Sorlie P. Some health benefits of physical activity. The Framingham Study. *Archives of internal medicine* 1979;139(8):857-61.

51. Palmer TM, Sterne JA, Harbord RM, et al. Instrumental variable estimation of causal risk ratios and causal odds ratios in Mendelian randomization analyses. *Am J Epidemiol* 2011;173(12):1392-403. doi: 10.1093/aje/kwr026 [published Online First: 2011/05/09]

52. Jaleel F, Jaleel A, Rahman MA, et al. Comparison of adiponectin, leptin and blood lipid levels in normal and obese postmenopausal women. *J Pak Med Assoc* 2006;56(9):391-4.

53. Kavazarakis E, Moustaki M, Gourgiotis D, et al. Relation of serum leptin levels to lipid profile in healthy children. *Metabolism* 2001;50(9):1091-4. doi: 10.1053/meta.2001.25606

54. Ayina CN, Noubiap JJ, Etoundi Ngoa LS, et al. Association of serum leptin and adiponectin with anthropomorphic indices of obesity, blood lipids and insulin resistance in a Sub-Saharan African population. *Lipids in health and disease* 2016;15:96. doi: 10.1186/s12944-016-0264-x

55. Jürimäe T, Jürimäe J, Leppik A, et al. Relationships between adiponectin, leptin, and blood lipids in physically active postmenopausal females. *Am J Hum Biol* 2010;22(5):609-12. doi: 10.1002/ajhb.21052

56. Hynes GR, Jones PJ. Leptin and its role in lipid metabolism. *Curr Opin Lipidol* 2001;12(3):321-7.

57. Singh P, Peterson TE, Sert-Kuniyoshi FH, et al. Leptin signaling in adipose tissue: role in lipid accumulation and weight gain. *Circ Res* 2012;111(5):599-603. doi: 10.1161/CIRCRESAHA.112.273656 [published Online First: 2012/06/22]

58. Gao L, Zhou Y, Zhong W, et al. Caveolin-1 is essential for protecting against binge drinking-induced liver damage through inhibiting reactive nitrogen species. *Hepatology* 2014;60(2):687-99. doi: 10.1002/hep.27162 [published Online First: 2014/05/19]

59. Haghighatdoost F, Hariri M. Effect of resveratrol on lipid profile: An updated systematic review and meta-analysis on randomized clinical trials. *Pharmacol Res* 2018;129:141-50. doi: 10.1016/j.phrs.2017.12.033 [published Online First: 2018/01/02]

60. Leng E, Xiao Y, Mo Z, et al. Synergistic effect of phytochemicals on cholesterol metabolism and lipid accumulation in HepG2 cells. *BMC Complement Altern Med* 2018;18(1):122. doi: 10.1186/s12906-018-2189-6 [published Online First: 2018/04/05]

Figure legends

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

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 (n=3,860)	Quartiles of the leptin GRS				
		Q1 (n=964)	Q2 (n=961)	Q3 (n=977)	Q4 (n=958)	*P*
Genetic risk score, mean (SD)	0.07 (0.03)	0.03(0.02)	0.06 (0.01)	0.09 (0.01)	0.11 (0.01)	
Age, years, mean (SD)	40.2 (8.9)	40.5 (8.7)	39.9 (8.8)	40.2 (9.1)	39.9 (8.8)	0.23
Male, N (%)	1808 (46.8)	453 (47.0)	437 (45.5)	460 (47.1)	458 (47.8)	0.89
Education levels, N (%)						
No more than high school	591 (15.4)	141 (14.7)	146 (15.3)	157 (16.1)	147 (15.4)	
Some college	1213 (31.5)	306 (31.8)	287 (30.0)	313 (32.1)	307 (32.3)	0.22
Bachelor's degree and above	2041 (53.1)	514 (53.5)	524 (54.8)	505 (51.8)	498 (52.3)	
Current Smoker, N (%)	603 (15.6)	144 (15.0)	152 (15.8)	165 (16.9)	142 (14.8)	0.53
Current Drinker, N (%)	3419 (89.1)	858 (89.4)	853 (89.1)	863 (88.9)	845 (89.0)	0.32
Physical Activities index score, mean(SD)	37.5 (7.9)	37.8 (8.0)	37.3 (8.1)	37.4 (7.7)	37.4 (7.8)	0.51
BMI, kg/m2, 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)	36.6 (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)	189.2 (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.

[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.
*Log transformed leptin and triglycerides were used to calculate the *P*-values.

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		$P_{interaction}$[b]	Fully adjusted model[c]		$P_{interaction}$[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 (18.36)	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 (50.13)	0.93	0.93
Current drinkers	-0.14 (17.10)	0.99		-1.80 (17.13)	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 (0.84)	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 (18.11)	0.49	
Not current drinkers	-15.20 (54.11)	0.78	0.96	-19.13 (55.06)	0.73	0.86
Current drinkers	-10.20 (19.37)	0.60		-11.43 (19.32)	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 GRS2[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		$P_{interaction}$[b]	Fully adjusted model[c]		$P_{interaction}$[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	

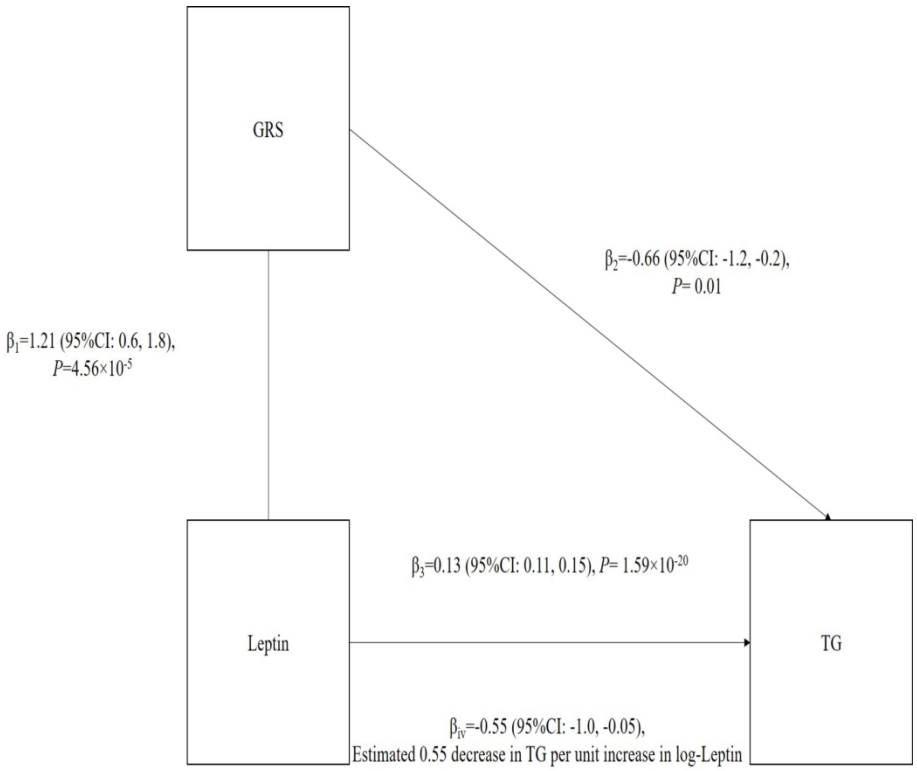
GRS=Genetic Risk Score; HDL-C =High-density lipoprotein cholesterol; LDL-C =Low-density lipoprotein cholesterol; log-TG=logarithmically transformed triglycerides; SE=standard error.

[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.



155x127mm (300 x 300 DPI)

Supplemental Table S1. Basic Information of three SNPs for BMI independent leptin GRS1[a] reaching genome-wide significance ($P<5\times10^{-8}$)

Chromosome Position	rsID	Coded Allele	Non-coded Allele	R^2	Function	Nearest 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 ($P<5\times10^{-8}$)

Chromosome Position	rsID	Coded Allele	Non-coded Allele	R^2	Function	Nearest Gene	Population
2:27742603	rs780093	C	T	0.995	intron variant	GCKR	European American
3:156798775	rs900400	T	C	0.691	upstream variant 2KB	CCDC80	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		$P_{interaction}$[b]	Fully adjusted model[c]		$P_{interaction}$[d]
	Beta(SE)	*P*		Beta(SE)	*P*	
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		$P_{interaction}$[b]	Fully adjusted model[c]		$P_{interaction}$[d]
	Beta(SE)	*P*		Beta(SE)	*P*	
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		$P_{interaction}$[b]	Fully adjusted model[c]		$P_{interaction}$[d]
	Beta(SE)	*P*		Beta(SE)	*P*	
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.

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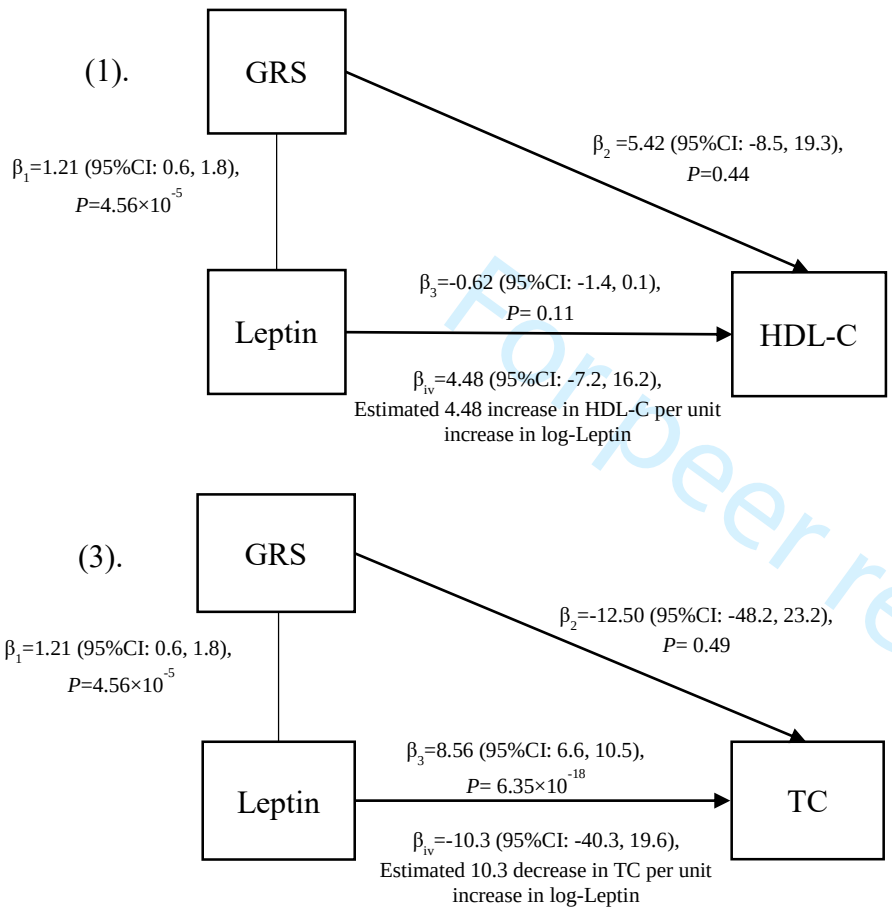
Supplemental Table S6. Association of BMI dependent Leptin GRS2[a] with Baseline Lipids among Overall, Drinking, and Non-Drinking Participants without treating for Lipids and Diabetes of the Framingham Heart Study 3rd Generation Cohort, respectively.

	Age, sex, BMI, waist adjusted model		$P_{interaction}$[b]	Fully adjusted model[c]		$P_{interaction}$[d]
	Beta(SE)	*P*		Beta(SE)	*P*	
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	
			0.06			0.08
Not current drinkers	-2.00 (0.75)	0.008		-1.87 (0.76)	0.01	

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	

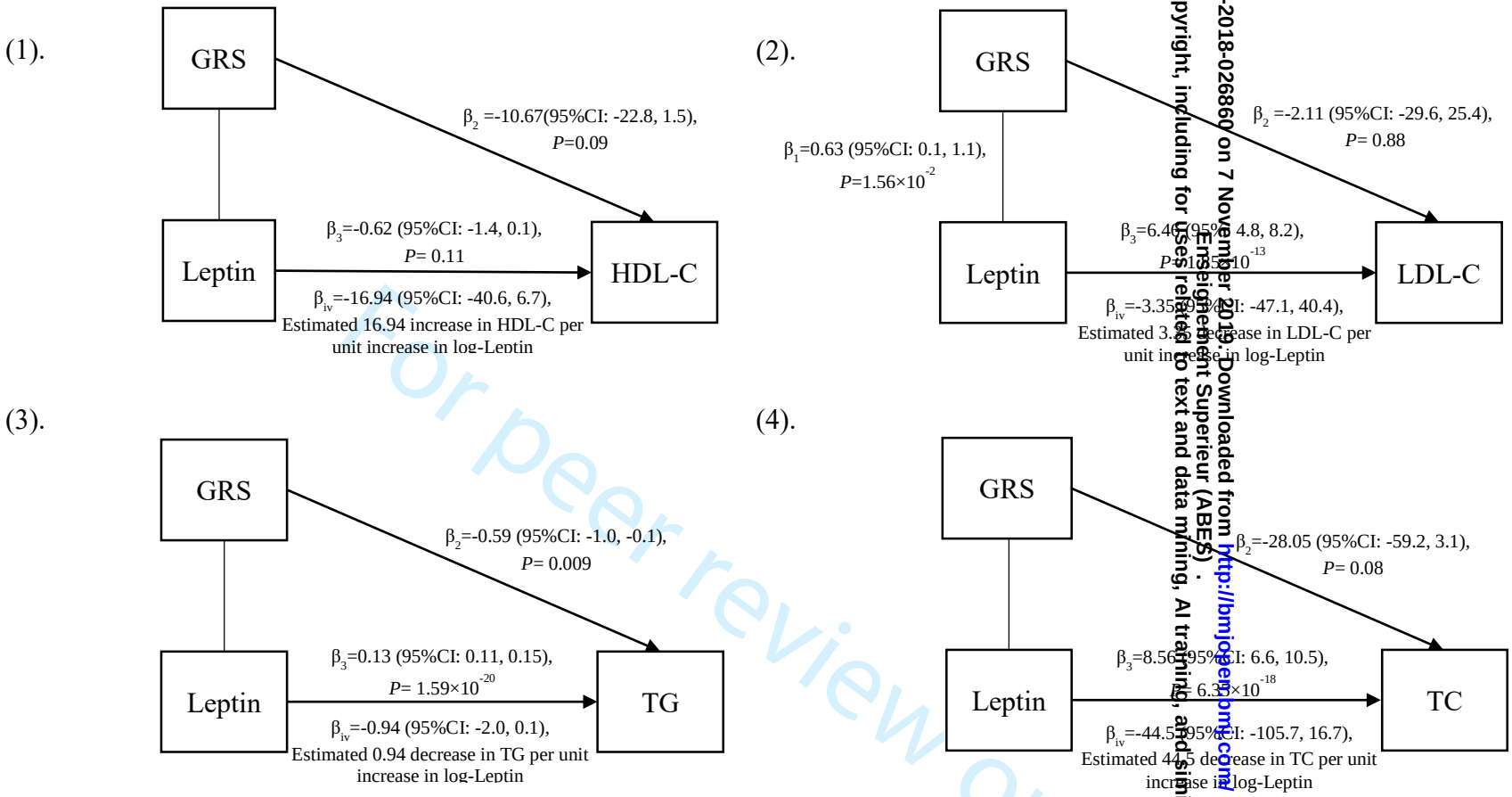
BMI=body mass index; GRS=Genetic Risk Score; HDL-C=High-density lipoprotein cholesterol; LDL-C=Low-density lipoprotein cholesterol; log-TG=logarithmically transformed triglycerides; SE=standard error

[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)

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

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

#6a	Cohort study – Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up. Case-control study – Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls. Cross-sectional study – Give the eligibility criteria, and the sources and methods of selection of participants. Give information on the criteria and methods for selection of subsets of participants from a larger study, when relevant.	5-6
#6b	Cohort study – For matched studies, give matching criteria and number of exposed and unexposed. Case-control study – For matched studies, give matching criteria and the number of controls per case.	n/a
#7a	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	7-8
#7b	Clearly define genetic exposures (genetic variants) using a widely-used nomenclature system. Identify variables likely to be associated with population stratification (confounding by ethnic origin).	6-7
#8a	For each variable of interest give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group. Give information separately for for exposed and unexposed groups if applicable.	5-6
#8b	Describe laboratory methods, including source and storage of DNA, genotyping methods and platforms (including the allele calling algorithm used, and its version), error rates and call rates. State the laboratory / centre where genotyping was done. Describe comparability of laboratory methods if there is more than one group. Specify whether genotypes were assigned using all of the data from the study simultaneously or in smaller batches.	6-7
#9a	Describe any efforts to address potential sources of bias	8-9
#9b	Describe any efforts to address potential sources of bias	8-9
#10	Explain how the study size was arrived at	5-6
#11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen, and why. If applicable, describe how effects of treatment were dealt with.	6
#12a	Describe all statistical methods, including those used to control for	8-9

	confounding. State software version used and options (or settings) chosen.	
#12b	Describe any methods used to examine subgroups and interactions	9
#12c	Explain how missing data were addressed	9
#12d	If applicable, explain how loss to follow-up was addressed	n/a
#12e	Describe any sensitivity analyses	9
#12f	State whether Hardy-Weinberg equilibrium was considered and, if so, how.	7
#12g	Describe any methods used for inferring genotypes or haplotypes	7
#12h	Describe any methods used to assess or address population stratification.	7
#12i	Describe any methods used to address multiple comparisons or to control risk of false positive findings.	7
#12j	Describe any methods used to address and correct for relatedness among subjects	n/a
#13a	Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed. Give information separately for for exposed and unexposed groups if applicable. Report numbers of individuals in whom genotyping was attempted and numbers of individuals in whom genotyping was successful.	5-6
#13b	Give reasons for non-participation at each stage	n/a
#13c	Consider use of a flow diagram	n/a
#14a	Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders. Give information separately for exposed and unexposed groups if applicable. Consider giving information by genotype	9-10
#14b	Indicate number of participants with missing data for each variable of interest	9-10
#14c	Cohort study – Summarize follow-up time, e.g. average and total amount.	n/a

#15	Cohort study Report numbers of outcome events or summary measures over time.Give information separately for exposed and unexposed groups if applicable. Report outcomes (phenotypes) for each genotype category over time Case-control study – Report numbers in each exposure category, or summary measures of exposure.Give information separately for cases and controls . Report numbers in each genotype category. Cross-sectional study – Report numbers of outcome events or summary measures. Give information separately for exposed and unexposed groups if applicable. Report outcomes (phenotypes) for each genotype category	9/10
#16a	Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	10-11
#16b	Report category boundaries when continuous variables were categorized	10-11
#16c	If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	10-11
#16d	Report results of any adjustments for multiple comparisons	10-11
#17a	Report other analyses done—e.g., analyses of subgroups and interactions, and sensitivity analyses	10-11
#17b	Report other analyses done—e.g., analyses of subgroups and interactions, and sensitivity analyses	10-11
#17c	Report other analyses done—e.g., analyses of subgroups and interactions, and sensitivity analyses	10-11
#18	Summarise key results with reference to study objectives	11-13
#19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias.	13-14
#20	Give a cautious overall interpretation considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence.	14
#21	Discuss the generalisability (external validity) of the study results	14
#22	Give the source of funding and the role of the funders for the present	15

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

BMJ Open

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

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

Running title: Genetically determined leptin and lipids

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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) (β=-0.66, p =0.01), but not low density lipoprotein cholesterol (LDL-C) (p=0.99), high density lipoprotein cholesterol (HDL-C) (p=0.44), or total cholesterol (TC) (p=0.49). Instrumental variable estimation showed that per unit increase in genetically determined log-leptin was associated with 0.55 (95% confidence interval: 0.05-1.00) units decrease in log-TG. Besides significant association with log-TG (β=-0.59, p=0.009), the BMI dependent GRS was nominally associated with HDL-C (β=-10.67, p=0.09) and TC (β=-28.05, p=0.08). When stratified by drinking status, the BMI dependent GRS was associated with reduced levels of LDL-C (p=0.03), log-TG (p=0.004), and TC (p=0.003) among non-current drinkers only. Significant interactions between the BMI dependent GRS and alcohol drinking were identified for LDL-C (p=0.03), 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.

Keywords: leptin, lipids, alcohol consumption, genetic risk score

Strengths and limitations of this study:

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

Introduction

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

leptin,[22] 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.[23-36] In rodent models, leptin has been demonstrated to be increased [26-28] or decreased [29 30] after alcohol intake. Similarly, leptin levels in human was decreased,[32] increased,[31 33] or even unchanged [34-36] after drinking. It is unclear whether alcohol consumption modifies the association of genetically determined leptin with lipid levels.[37 38]

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

Materials and Methods

Data Sources and Study Participants

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

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

Genotyping and Genetic Risk Score

Genetic loci for circulating leptin levels have been reported in a large genome-wide association studies (GWAS) meta-analysis by Kilpelainen and colleagues.[22] This study included 32,161 individuals of European ancestry and identified three single-nucleotide polymorphisms (SNPs), *GCKR* rs780093, *LEP* rs10487505, and *SLC32A1* rs6071166, that were robustly associated with body mass index (BMI) adjusted leptin at a genome-wide significance level ($p<5\times10^{-08}$). In addition, *GCKR* rs780093, *CCNL1* rs900400, and *FTO* rs8043757 were associated with circulating leptin without adjustment for BMI.[22] We assumed the additive genetic model for each SNP and constructed two genetic risk scores (GRSs) for leptin by combining leptin-increasing alleles for SNPs weighted by their corresponding effect sizes on logarithmically transformed leptin (log-leptin) as reported in the original GWAS meta-analysis.[22] The first score, GRS1, was generated using the three SNPs associated with BMI adjusted leptin, and the second score, GRS2, using the three SNPs associated with leptin unadjusted for BMI.

Genome-wide SNPs were genotyped using Affymetrix and Illumina platforms in the FHS. The 1000 Genome genotype data for the FHS was already imputed and cataloged on the dbGaP. According to the document of the FHS,[40] before imputation, quality control removed SNPs with a Hardy-Weinberg equilibrium $p<1\times10^{-6}$, a missing rate>3.1%, a minor allele frequency (MAF)<1%, a missing physical position or cannot mapped to build 37 positions, Mendelian errors > 1000, or duplicate SNPs. MACH software was used for genotype phasing, followed by imputation using MiniMac software.[41 42] Imputation results were summarized as

dosage scores, which represent the expected numbers of copies of the coded allele for each SNP, ranging from 0 to 2. After imputation, SNPs with r^2<0.30, an MAF<1%, or a Hardy-Weinberg equilibrium $p<1\times10^{-6}$ were removed. We retrieved genotypes of the SNPs for GRSs from the imputed data for all study participants (**Supplemental Table S1 and Supplemental Table S2**).

Leptin and Lipids measurement

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

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

Covariates

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

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

Statistical Analysis

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

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

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

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

Participant and Public Involvement

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

Results

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

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

BMI independent leptin GRS1 and blood lipids

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

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

BMI dependent leptin GRS2 and blood lipids

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

When restricting to participants not taking lipid-lowering medication and those not taking lipid- or glucose-lowering medications, respectively, the associations of GRS1 and GRS2 with blood lipids were similar to those as shown above (**Supplemental Table S3, S4, S5** and S**6**).

Discussion

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

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

Both the BMI dependent- and independent- GRSs were associated with lower level of log-TG in the current study. Inconsistent associations between leptin and blood lipids have been observed in previous studies. In a small study of 80 postmenopausal women, serum leptin was positively associated with HDL-C, TG, and TC, and inversely associated with LDL-C.[48] Another study conducted with 294 healthy school children reported that leptin was only associated with increased TG.[49] However, a study of 476 residents from Cameroon reported a positive correlation between leptin, LDL-C, and TC, and a positive association between leptin and TC, but no association between leptin and HDL-C or TG.[50] In a more recent study of 134 physically active postmenopausal women, no significant correlation was detected for leptin and blood lipids.[51] The divergent results of previous studies make it impossible to infer a relationship between leptin and blood lipids. Possible reasons for the divergent findings include varying sample sizes, failure to account for residual and unmeasured confounding, and the genetic background of the study population. Through Mendelian randomization analyses, we demonstrated that genetically determined leptin was inversely associated with log-TG. It is well known that alleles, such as risk alleles for leptin, are randomly assigned at meiosis and therefore, are independent of non-genetic confounders. The association between leptin GRS and log-TG in the current study was less prone to confounding. This also highlights the importance of using Mendelian randomization to delineate causal relationships. Our finding is further supported by previous physiologic studies, among which, leptin was demonstrated to inhibit lipogenesis, stimulate lipolysis, and reduce triglyceride uptake.[52] However, the association of HDL-C and TC

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

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

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

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

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

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Author Contributions

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

Conflict of interest

Conflicts of interest and disclosures: none.

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Data sharing statement

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

References

1. Campfield LA, Smith FJ, Guisez Y, et al. Recombinant mouse OB protein: evidence for a peripheral signal linking adiposity and central neural networks. *Science* 1995;269(5223):546-9.
2. Halaas JL, Gajiwala KS, Maffei M, et al. Weight-reducing effects of the plasma protein encoded by the obese gene. *Science* 1995;269(5223):543-6.
3. Zhang Y, Proenca R, Maffei M, et al. Positional cloning of the mouse obese gene and its human homologue. *Nature* 1994;372(6505):425-32. doi: 10.1038/372425a0
4. Sainz N, Barrenetxe J, Moreno-Aliaga MJ, et al. Leptin resistance and diet-induced obesity: central and peripheral actions of leptin. *Metabolism: clinical and experimental* 2015;64(1):35-46. doi: 10.1016/j.metabol.2014.10.015
5. Wang MY, Lee Y, Unger RH. Novel form of lipolysis induced by leptin. *The Journal of biological chemistry* 1999;274(25):17541-4.
6. Harris RB. Direct and indirect effects of leptin on adipocyte metabolism. *Biochimica et biophysica acta* 2014;1842(3):414-23. doi: 10.1016/j.bbadis.2013.05.009
7. Enser M, Ashwell M. Fatty acid composition of triglycerides from adipose tissue transplanted between obese and lean mice. *Lipids* 1983;18(11):776-80.
8. Selenscig D, Rossi A, Chicco A, et al. Increased leptin storage with altered leptin secretion from adipocytes of rats with sucrose-induced dyslipidemia and insulin resistance: effect of dietary fish oil. *Metabolism: clinical and experimental* 2010;59(6):787-95. doi: 10.1016/j.metabol.2009.09.025
9. Kosztaczky B, Foris G, Paragh G, Jr., et al. Leptin stimulates endogenous cholesterol synthesis in human monocytes: New role of an old player in atherosclerotic plaque

formation. Leptin-induced increase in cholesterol synthesis. *The international journal of biochemistry & cell biology* 2007;39(9):1637-45. doi: 10.1016/j.biocel.2007.04.012

10. Francisco V, Pino J, Campos-Cabaleiro V, et al. Obesity, Fat Mass and Immune System: Role for Leptin. *Front Physiol* 2018;9:640. doi: 10.3389/fphys.2018.00640 [published Online First: 2018/06/01]

11. La Cava A. Leptin in inflammation and autoimmunity. *Cytokine* 2017;98:51-58. doi: 10.1016/j.cyto.2016.10.011

12. Maurya R, Bhattacharya P, Dey R, et al. Leptin Functions in Infectious Diseases. *Front Immunol* 2018;9:2741. doi: 10.3389/fimmu.2018.02741 [published Online First: 2018/11/26]

13. Kakuma T, Lee Y, Higa M, et al. Leptin, troglitazone, and the expression of sterol regulatory element binding proteins in liver and pancreatic islets. *Proc Natl Acad Sci U S A* 2000;97(15):8536-41. doi: 10.1073/pnas.97.15.8536

14. Im SS, Yousef L, Blaschitz C, et al. Linking lipid metabolism to the innate immune response in macrophages through sterol regulatory element binding protein-1a. *Cell Metab* 2011;13(5):540-9. doi: 10.1016/j.cmet.2011.04.001

15. Paz-Filho G, Mastronardi CA, Licinio J. Leptin treatment: facts and expectations. *Metabolism: clinical and experimental* 2015;64(1):146-56. doi: 10.1016/j.metabol.2014.07.014

16. Park JY, Javor ED, Cochran EK, et al. Long-term efficacy of leptin replacement in patients with Dunnigan-type familial partial lipodystrophy. *Metabolism: clinical and experimental* 2007;56(4):508-16. doi: 10.1016/j.metabol.2006.11.010

17. Javor ED, Cochran EK, Musso C, et al. Long-term efficacy of leptin replacement in patients with generalized lipodystrophy. *Diabetes* 2005;54(7):1994-2002.

18. Ebihara K, Kusakabe T, Hirata M, et al. Efficacy and safety of leptin-replacement therapy and possible mechanisms of leptin actions in patients with generalized lipodystrophy. *The Journal of clinical endocrinology and metabolism* 2007;92(2):532-41. doi: 10.1210/jc.2006-1546

19. Kamran F, Rother KI, Cochran E, et al. Consequences of stopping and restarting leptin in an adolescent with lipodystrophy. *Hormone research in paediatrics* 2012;78(5-6):320-5. doi: 10.1159/000341398

20. Wu DM, Shen MH, Chu NF. Relationship between plasma leptin levels and lipid profiles among school children in Taiwan--the Taipei Children Heart Study. *European journal of epidemiology* 2001;17(10):911-6.

21. Sekhar RV, Jahoor F, Iyer D, et al. Leptin replacement therapy does not improve the abnormal lipid kinetics of hypoleptinemic patients with HIV-associated lipodystrophy syndrome. *Metabolism* 2012;61(10):1395-403. doi: 10.1016/j.metabol.2012.03.013 [published Online First: 2012/04/28]

22. Kilpelainen TO, Carli JF, Skowronski AA, et al. Genome-wide meta-analysis uncovers novel loci influencing circulating leptin levels. *Nature communications* 2016;7:10494. doi: 10.1038/ncomms10494

23. Rojdmark S, Calissendorff J, Brismar K. Alcohol ingestion decreases both diurnal and nocturnal secretion of leptin in healthy individuals. *Clinical endocrinology* 2001;55(5):639-47.

24. Roth MJ, Baer DJ, Albert PS, et al. Relationship between serum leptin levels and alcohol consumption in a controlled feeding and alcohol ingestion study. *Journal of the National Cancer Institute* 2003;95(22):1722-5.

25. Otaka M, Konishi N, Odashima M, et al. Effect of alcohol consumption on leptin level in serum, adipose tissue, and gastric mucosa. *Digestive diseases and sciences* 2007;52(11):3066-9. doi: 10.1007/s10620-006-9635-x

26. Pravdova E, Macho L, Fickova M. Alcohol intake modifies leptin, adiponectin and resistin serum levels and their mRNA expressions in adipose tissue of rats. *Endocrine regulations* 2009;43(3):117-25.

27. Slomiany BL, Slomiany A. Role of epidermal growth factor receptor transactivation in the activation of cytosolic phospholipase A(2) in leptin protection of salivary gland acinar cells against ethanol cytotoxicity. *Journal of physiology and pharmacology : an official journal of the Polish Physiological Society* 2009;60(2):49-55.

28. Yu HC, Li SY, Cao MF, et al. Effects of chronic ethanol consumption on levels of adipokines in visceral adipose tissues and sera of rats. *Acta pharmacologica Sinica* 2010;31(4):461-9. doi: 10.1038/aps.2010.12

29. Maddalozzo GF, Turner RT, Edwards CH, et al. Alcohol alters whole body composition, inhibits bone formation, and increases bone marrow adiposity in rats. *Osteoporosis international : a journal established as result of cooperation between the European Foundation for Osteoporosis and the National Osteoporosis Foundation of the USA* 2009;20(9):1529-38. doi: 10.1007/s00198-009-0836-y

30. Tan X, Sun X, Li Q, et al. Leptin deficiency contributes to the pathogenesis of alcoholic fatty liver disease in mice. *The American journal of pathology* 2012;181(4):1279-86. doi: 10.1016/j.ajpath.2012.06.013

31. Henriksen JH, Holst JJ, Moller S, et al. Increased circulating leptin in alcoholic cirrhosis: relation to release and disposal. *Hepatology* 1999;29(6):1818-24. doi: 10.1002/hep.510290601

32. Santolaria F, Perez-Cejas A, Aleman MR, et al. Low serum leptin levels and malnutrition in chronic alcohol misusers hospitalized by somatic complications. *Alcohol and alcoholism* 2003;38(1):60-6.

33. Nicolas JM, Fernandez-Sola J, Fatjo F, et al. Increased circulating leptin levels in chronic alcoholism. *Alcoholism, clinical and experimental research* 2001;25(1):83-8.

34. Beulens JW, de Zoete EC, Kok FJ, et al. Effect of moderate alcohol consumption on adipokines and insulin sensitivity in lean and overweight men: a diet intervention study. *European journal of clinical nutrition* 2008;62(9):1098-105. doi: 10.1038/sj.ejcn.1602821

35. Voican CS, Njike-Nakseu M, Boujedidi H, et al. Alcohol withdrawal alleviates adipose tissue inflammation in patients with alcoholic liver disease. *Liver international : official journal of the International Association for the Study of the Liver* 2015;35(3):967-78. doi: 10.1111/liv.12575

36. Greco AV, Mingrone G, Favuzzi A, et al. Serum leptin levels in post-hepatitis liver cirrhosis. *Journal of hepatology* 2000;33(1):38-42.

37. Wannamethee SG, Tchernova J, Whincup P, et al. Plasma leptin: associations with metabolic, inflammatory and haemostatic risk factors for cardiovascular disease. *Atherosclerosis* 2007;191(2):418-26. doi: 10.1016/j.atherosclerosis.2006.04.012

38. Balasubramaniyan V, Nalini N. Intraperitoneal leptin regulates lipid metabolism in ethanol supplemented Mus musculas heart. *Life sciences* 2006;78(8):831-7. doi: 10.1016/j.lfs.2005.05.079

39. Splansky GL, Corey D, Yang Q, et al. The Third Generation Cohort of the National Heart, Lung, and Blood Institute's Framingham Heart Study: design, recruitment, and initial examination. *American journal of epidemiology* 2007;165(11):1328-35. doi: 10.1093/aje/kwm021

40. 1000 Genome imputation based on the November 2010 release 1000 genome imputation general procedure, 2010.

41. Auton A, Brooks LD, Durbin RM, et al. A global reference for human genetic variation. *Nature* 2015;526(7571):68-74. doi: 10.1038/nature15393

42. Das S, Forer L, Schönherr S, et al. Next-generation genotype imputation service and methods. *Nat Genet* 2016;48(10):1284-7. doi: 10.1038/ng.3656 [published Online First: 2016/08/29]

43. Andersson C, Lyass A, Larson MG, et al. Low-density-lipoprotein cholesterol concentrations and risk of incident diabetes: epidemiological and genetic insights from the Framingham Heart Study. *Diabetologia* 2015;58(12):2774-80. doi: 10.1007/s00125-015-3762-x

44. Rao DC, Sung YJ, Winkler TW, et al. Multiancestry Study of Gene-Lifestyle Interactions for Cardiovascular Traits in 610 475 Individuals From 124 Cohorts: Design and Rationale. *Circ Cardiovasc Genet* 2017;10(3) doi: 10.1161/CIRCGENETICS.116.001649

45. Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. *Clinical chemistry* 1972;18(6):499-502.

46. Kannel WB, Sorlie P. Some health benefits of physical activity. The Framingham Study. *Archives of internal medicine* 1979;139(8):857-61.

47. Palmer TM, Sterne JA, Harbord RM, et al. Instrumental variable estimation of causal risk ratios and causal odds ratios in Mendelian randomization analyses. *Am J Epidemiol* 2011;173(12):1392-403. doi: 10.1093/aje/kwr026 [published Online First: 2011/05/09]

48. Jaleel F, Jaleel A, Rahman MA, et al. Comparison of adiponectin, leptin and blood lipid levels in normal and obese postmenopausal women. *J Pak Med Assoc* 2006;56(9):391-4.

49. Kavazarakis E, Moustaki M, Gourgiotis D, et al. Relation of serum leptin levels to lipid profile in healthy children. *Metabolism* 2001;50(9):1091-4. doi: 10.1053/meta.2001.25606

50. Ayina CN, Noubiap JJ, Etoundi Ngoa LS, et al. Association of serum leptin and adiponectin with anthropomorphic indices of obesity, blood lipids and insulin resistance in a Sub-Saharan African population. *Lipids in health and disease* 2016;15:96. doi: 10.1186/s12944-016-0264-x

51. Jürimäe T, Jürimäe J, Leppik A, et al. Relationships between adiponectin, leptin, and blood lipids in physically active postmenopausal females. *Am J Hum Biol* 2010;22(5):609-12. doi: 10.1002/ajhb.21052

52. Hynes GR, Jones PJ. Leptin and its role in lipid metabolism. *Curr Opin Lipidol* 2001;12(3):321-7.

53. Singh P, Peterson TE, Sert-Kuniyoshi FH, et al. Leptin signaling in adipose tissue: role in lipid accumulation and weight gain. *Circ Res* 2012;111(5):599-603. doi: 10.1161/CIRCRESAHA.112.273656 [published Online First: 2012/06/22]

54. Gao L, Zhou Y, Zhong W, et al. Caveolin-1 is essential for protecting against binge drinking-induced liver damage through inhibiting reactive nitrogen species. *Hepatology* 2014;60(2):687-99. doi: 10.1002/hep.27162 [published Online First: 2014/05/19]

55. Haghighatdoost F, Hariri M. Effect of resveratrol on lipid profile: An updated systematic review and meta-analysis on randomized clinical trials. *Pharmacol Res* 2018;129:141-50. doi: 10.1016/j.phrs.2017.12.033 [published Online First: 2018/01/02]

56. Leng E, Xiao Y, Mo Z, et al. Synergistic effect of phytochemicals on cholesterol metabolism and lipid accumulation in HepG2 cells. *BMC Complement Altern Med* 2018;18(1):122. doi: 10.1186/s12906-018-2189-6 [published Online First: 2018/04/05]

Figure legends

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

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 (n=3,860)	Quartiles of the leptin GRS				
		Q1 (n=964)	Q2 (n=961)	Q3 (n=977)	Q4 (n=958)	*P*
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)	40.2 (9.1)	39.9 (8.8)	0.23
Male, N (%)	1808 (46.8)	453 (47.0)	437 (45.5)	460 (47.1)	458 (47.8)	0.89
Education levels, N (%)						
No more than high school	591 (15.4)	141 (14.7)	146 (15.3)	157 (16.1)	147 (15.4)	
Some college	1213 (31.5)	306 (31.8)	287 (30.0)	313 (32.1)	307 (32.3)	0.22
Bachelor's degree and above	2041 (53.1)	514 (53.5)	524 (54.8)	505 (51.8)	498 (52.3)	
Current Smoker, N (%)	603 (15.6)	144 (15.0)	152 (15.8)	165 (16.9)	142 (14.8)	0.53
Current Drinker, N (%)	3419 (89.1)	858 (89.4)	853 (89.1)	863 (88.9)	845 (89.0)	0.32
Physical Activities index score, mean(SD)	37.5 (7.9)	37.8 (8.0)	37.3 (8.1)	37.4 (7.7)	37.4 (7.8)	0.51
BMI, kg/m2, 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)	36.6 (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)	189.2 (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.

[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.
*Log transformed leptin and triglycerides were used to calculate the *P*-values.

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		$P_{interaction}$[b]	Fully adjusted model[c]		$P_{interaction}$[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 (18.36)	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 (50.13)	0.93	0.93
Current drinkers	-0.14 (17.10)	0.99		-1.80 (17.13)	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 (0.84)	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 (18.11)	0.49	
Not current drinkers	-15.20 (54.11)	0.78	0.96	-19.13 (55.06)	0.73	0.86
Current drinkers	-10.20 (19.37)	0.60		-11.43 (19.32)	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 GRS2[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		$P_{interaction}$[b]	Fully adjusted model[c]		$P_{interaction}$[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	

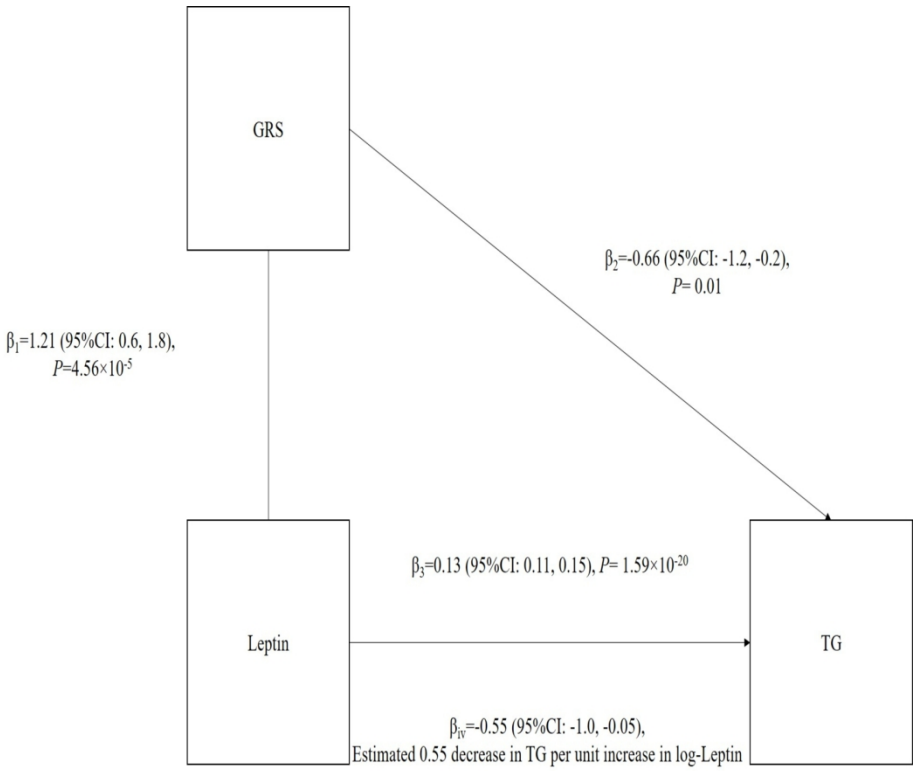
GRS=Genetic Risk Score; HDL-C =High-density lipoprotein cholesterol; LDL-C =Low-density lipoprotein cholesterol; log-TG=logarithmically transformed triglycerides; SE=standard error.

[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 ($P<5\times10^{-8}$)

Chromosome Position	rsID	Coded Allele	Non-coded Allele	R^2	Function	Nearest 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 ($P<5\times10^{-8}$)

Chromosome Position	rsID	Coded Allele	Non-coded Allele	R^2	Function	Nearest Gene	Population
2:27742603	rs780093	C	T	0.995	intron variant	GCKR	European American
3:156798775	rs900400	T	C	0.691	upstream variant 2KB	CCDC80	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		$P_{interaction}$[b]	Fully adjusted model[c]		$P_{interaction}$[d]
	Beta(SE)	*P*		Beta(SE)	*P*	
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		$P_{interaction}$[b]	Fully adjusted model[c]		$P_{interaction}$[d]
	Beta(SE)	*P*		Beta(SE)	*P*	
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		$P_{interaction}$[b]	Fully adjusted model[c]		$P_{interaction}$[d]
	Beta(SE)	*P*		Beta(SE)	*P*	
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.

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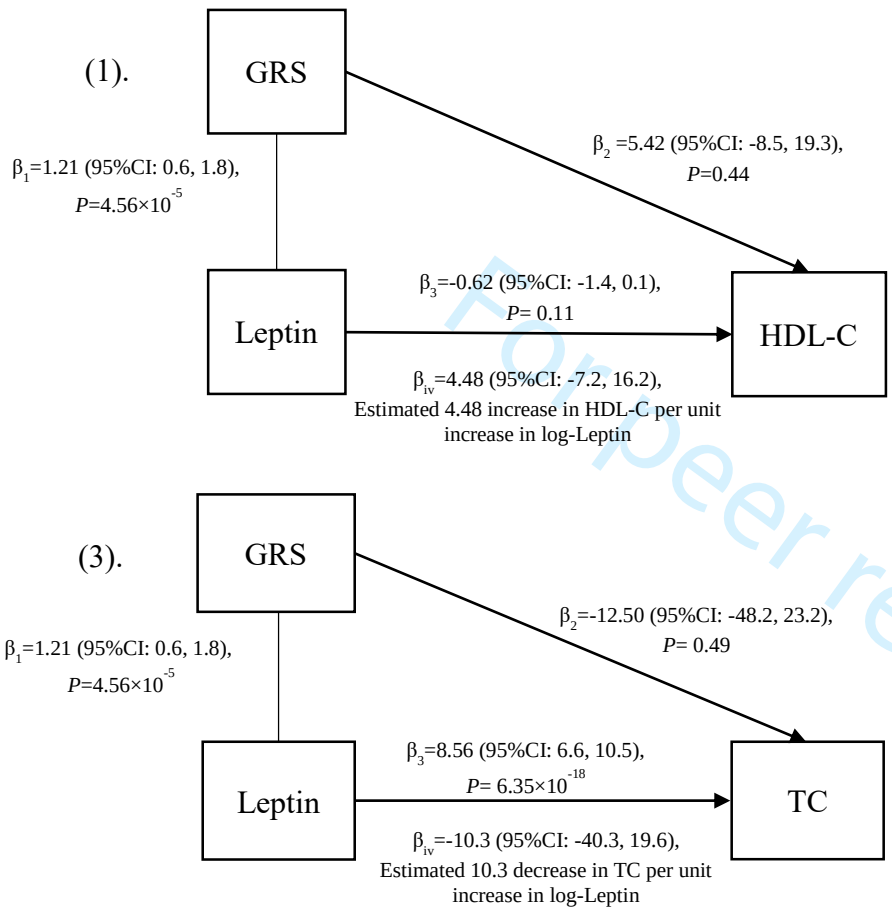
Supplemental Table S6. Association of BMI dependent Leptin GRS2[a] with Baseline Lipids among Overall, Drinking, and Non-Drinking Participants without treating for Lipids and Diabetes of the Framingham Heart Study 3rd Generation Cohort, respectively.

	Age, sex, BMI, waist adjusted model		$P_{interaction}$[b]	Fully adjusted model[c]		$P_{interaction}$[d]
	Beta(SE)	*P*		Beta(SE)	*P*	
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	
			0.06			0.08
Not current drinkers	-2.00 (0.75)	0.008		-1.87 (0.76)	0.01	

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	

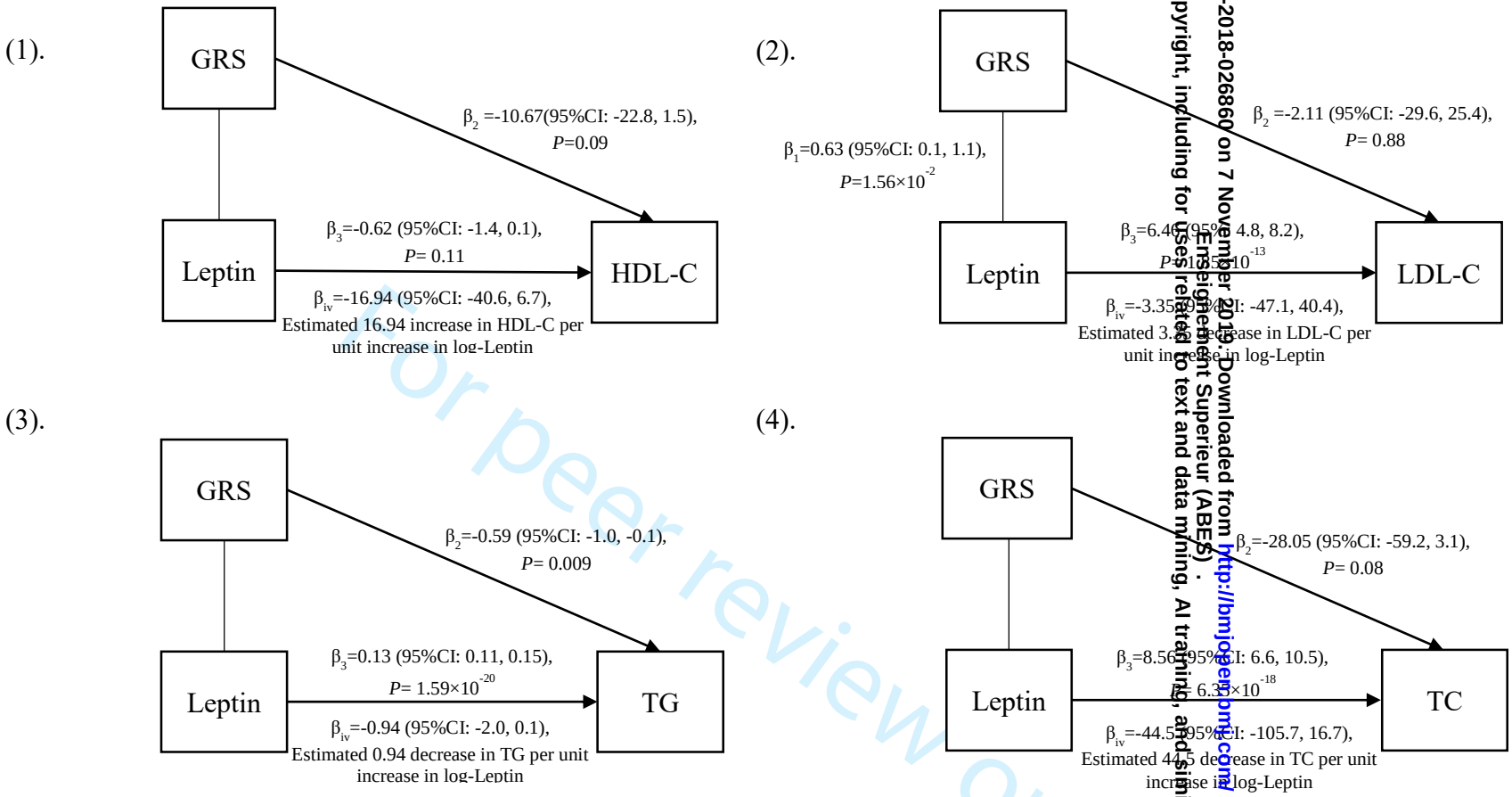
BMI=body mass index; GRS=Genetic Risk Score; HDL-C=High-density lipoprotein cholesterol; LDL-C=Low-density lipoprotein cholesterol; log-TG=logarithmically transformed triglycerides; SE=standard error

[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)

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

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

#6a	Cohort study – Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up. Case-control study – Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls. Cross-sectional study – Give the eligibility criteria, and the sources and methods of selection of participants. Give information on the criteria and methods for selection of subsets of participants from a larger study, when relevant.	5-6
#6b	Cohort study – For matched studies, give matching criteria and number of exposed and unexposed. Case-control study – For matched studies, give matching criteria and the number of controls per case.	n/a
#7a	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	7-8
#7b	Clearly define genetic exposures (genetic variants) using a widely-used nomenclature system. Identify variables likely to be associated with population stratification (confounding by ethnic origin).	6-7
#8a	For each variable of interest give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group. Give information separately for for exposed and unexposed groups if applicable.	5-6
#8b	Describe laboratory methods, including source and storage of DNA, genotyping methods and platforms (including the allele calling algorithm used, and its version), error rates and call rates. State the laboratory / centre where genotyping was done. Describe comparability of laboratory methods if there is more than one group. Specify whether genotypes were assigned using all of the data from the study simultaneously or in smaller batches.	6-7
#9a	Describe any efforts to address potential sources of bias	8-9
#9b	Describe any efforts to address potential sources of bias	8-9
#10	Explain how the study size was arrived at	5-6
#11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen, and why. If applicable, describe how effects of treatment were dealt with.	6
#12a	Describe all statistical methods, including those used to control for	8-9

	confounding. State software version used and options (or settings) chosen.	
#12b	Describe any methods used to examine subgroups and interactions	9
#12c	Explain how missing data were addressed	9
#12d	If applicable, explain how loss to follow-up was addressed	n/a
#12e	Describe any sensitivity analyses	9
#12f	State whether Hardy-Weinberg equilibrium was considered and, if so, how.	7
#12g	Describe any methods used for inferring genotypes or haplotypes	7
#12h	Describe any methods used to assess or address population stratification.	7
#12i	Describe any methods used to address multiple comparisons or to control risk of false positive findings.	7
#12j	Describe any methods used to address and correct for relatedness among subjects	n/a
#13a	Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed. Give information separately for for exposed and unexposed groups if applicable. Report numbers of individuals in whom genotyping was attempted and numbers of individuals in whom genotyping was successful.	5-6
#13b	Give reasons for non-participation at each stage	n/a
#13c	Consider use of a flow diagram	n/a
#14a	Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders. Give information separately for exposed and unexposed groups if applicable. Consider giving information by genotype	9-10
#14b	Indicate number of participants with missing data for each variable of interest	9-10
#14c	Cohort study – Summarize follow-up time, e.g. average and total amount.	n/a

#15	Cohort study Report numbers of outcome events or summary measures over time.Give information separately for exposed and unexposed groups if applicable. Report outcomes (phenotypes) for each genotype category over time Case-control study – Report numbers in each exposure category, or summary measures of exposure.Give information separately for cases and controls . Report numbers in each genotype category. Cross-sectional study – Report numbers of outcome events or summary measures. Give information separately for exposed and unexposed groups if applicable. Report outcomes (phenotypes) for each genotype category	9/10
#16a	Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	10-11
#16b	Report category boundaries when continuous variables were categorized	10-11
#16c	If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	10-11
#16d	Report results of any adjustments for multiple comparisons	10-11
#17a	Report other analyses done—e.g., analyses of subgroups and interactions, and sensitivity analyses	10-11
#17b	Report other analyses done—e.g., analyses of subgroups and interactions, and sensitivity analyses	10-11
#17c	Report other analyses done—e.g., analyses of subgroups and interactions, and sensitivity analyses	10-11
#18	Summarise key results with reference to study objectives	11-13
#19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias.	13-14
#20	Give a cautious overall interpretation considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence.	14
#21	Discuss the generalisability (external validity) of the study results	14
#22	Give the source of funding and the role of the funders for the present	15

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