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# **BMJ Open**

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

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



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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	ġ
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Abstract

2	Objectives: We evaluated the effect of genetically determined leptin on lipids using baseline
3	data for 3,860 participants of the Framingham Heart Study 3 <sup>rd</sup> Generation cohort.
4	Material and methods: Two genetic risk scores (GRSs) were generated using leptin
5	loci independent and dependent of body mass index (BMI), respectively. Associations between
6	leptin GRSs and leptin levels, leptin and lipid levels, and the leptin GRSs and lipid levels were
7	assessed by multivariate linear regression models. Interactions between the GRSs and alcohol
8	consumption were also evaluated in the models.
9	Results: Both GRSs were positively associated with log transformed leptin (log-leptin). The
10	BMI independent leptin GRS was associated with log transformed triglycerides (log-TG) ( $\beta$ =-
11	0.66, $p$ =0.01), but not low density lipoprotein cholesterol (LDL-C) ( $p$ =0.99), high density
12	lipoprotein cholesterol (HDL-C) ( $p=0.44$ ), or total cholesterol (TC) ( $p=0.49$ ). Instrumental
13	variable estimation showed that per unit increase in genetically determined log-leptin was
14	associated with 0.55 (95% confidence interval: 0.05-1.00) units decrease in log-TG. Besides
15	significant association with log-TG ( $\beta$ =-0.59, p=0.009), the BMI dependent GRS was nominally
16	associated with HDL-C ( $\beta$ =-10.67, <i>p</i> =0.09) and TC ( $\beta$ =-28.05, <i>p</i> =0.08). When stratified by
17	drinking status, the BMI dependent GRS was associated with reduced levels of LDL-C (p=0.03),
18	log-TG (p=0.004), and TC (p=0.003) among non-current drinkers only. Significant interactions
19	between the BMI dependent GRS and alcohol drinking were identified for LDL-C ( $p=0.03$ ), TG
20	(p=0.03), and TC $(p=0.02)$ .

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<b>Conclusion:</b> 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:
<ul> <li>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.</li> <li>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.</li> <li>Pleiotropy effects of SNPs included in the leptin genetic risk score (GRS) may confound the leptin GRS and lipids associations.</li> <li>Our analyses were restricted to individuals of European ancestry.</li> </ul>
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### 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 

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1	(Beulens et al., 2008, Greco et al., 2000, Henriksen et al., 1999, Maddalozzo et al., 2009, Nicolas
2	et al., 2001, Otaka et al., 2007, Pravdova et al., 2009, Rojdmark et al., 2001, Roth et al., 2003,
3	Santolaria et al., 2003, Slomiany and Slomiany, 2009, Tan et al., 2012, Voican et al., 2015, Yu et
4	al., 2010). In rodent models, leptin has been demonstrated to be increased (Pravdova et al.,
5	2009, Slomiany and Slomiany, 2009, Yu et al., 2010) or decreased (Maddalozzo et al., 2009, Tan
6	et al., 2012) after alcohol intake. Similarly, leptin levels in human was decreased (Santolaria et
7	al., 2003), increased(Henriksen et al., 1999, Nicolas et al., 2001), or even unchanged (Beulens et
8	al., 2008, Greco et al., 2000, Voican et al., 2015) after drinking. It is unclear whether alcohol
9	consumption modifies the effect of genetically determined leptin on lipid levels
10	(Balasubramaniyan and Nalini, 2006, Wannamethee et al., 2007).
11	Therefore, the objectives of the current study were to evaluate the relationship between
12	genetically determined leptin and lipid levels and to explore whether the leptin-lipids
13	associations could be modified by alcohol consumption among participants of the Framingham
14	Heart Study (FHS) 3 <sup>rd</sup> generation cohort.
15	Materials and Methods
16	Data Sources and Study Participants
17	The FHS was designed to identify common factors or characteristics that contribute to
18	cardiovascular disease (CVD) by tracking the development of CVD over a long period of time.
19	Participants of the FHS were free from overt symptoms of CVD or stroke at baseline. Later on,
20	the FHS was extended to including offspring and third generation of the original participants. A
21	detailed description of the FHS 3 <sup>rd</sup> generation cohort has been outlined in previous publications
22	(Splansky et al., 2007). Genotype and phenotype data of the FHS are cataloged on the database

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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 3<sup>rd</sup> 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 ( $p < 5 \times 10^{-08}$ ). 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  $p < 1 \times 10^{-6}$ , 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  $p < 1 \times 10^{-6}$  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.

### 21 Covariates

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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. 

### 11 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 logleptin 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 Page 9 of 46

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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</li>
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%

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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 <sup>2</sup> 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
( <i>p</i> =0.53), drinking ( <i>p</i> =0.32), BMI ( <i>p</i> =0.94), waist circumference ( <i>p</i> =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 ( $p=4.56 \times 10^{-5}$ ).

### 10 BMI independent leptin GRS1 and blood lipids

After controlling for age, sex, BMI, and waist circumference, log-leptin was positively associated with TC ( $\beta$ =8.56, p=6.35×10<sup>-18</sup>), LDL-C ( $\beta$ =6.46, p=1.85×10<sup>-13</sup>), and log-TG ( $\beta$ =0.13,  $p=1.59\times10^{-20}$ ), but was not associated with HDL-C ( $\beta=-0.62$ , 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 ( $\beta$ =-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 ( $\beta$ =-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 ( $\beta$ =-12.50, p=0.49) and LDL-C ( $\beta$ =-0.11, p=0.99) and positively associated with HDL-C ( $\beta=5.42$ , p=0.44), however, the correlations were

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1	not significant. The GRS1 and blood lipids associations were not modified by drinking status
2	(Table 2).
3	BMI dependent leptin GRS2 and blood lipids
4	As expected, the BMI dependent leptin GRS2 was not associated with any covariate
5	except for the BMI ( $p=0.02$ ) and waist circumference ( $p=0.03$ ). In the analyses controlling for
6	age, sex, BMI, and waist circumference, the GRS2 was significantly associated with lower levels
7	of log-TG ( $p$ =0.009) and nominally associated with lower levels of HDL-C ( $p$ =0.09) and TC
8	(p=0.08) (Supplemental Figure S2). When stratified by drinking status, the leptin GRS2 was
9	negatively associated with LDL-C ( $\beta$ =-92.51, p=0.03), log-TG ( $\beta$ =-2.07, p=0.004), and TC ( $\beta$ =-
10	144.68, <i>p</i> =0.003) only among non-current drinkers ( <b>Table 3</b> ). When further adjusting for
11	education, smoking, drinking, and physical activity, those associations persisted (Table 3).
12	Furthermore, significant interactions between leptin GRS2 and alcohol drinking were identified
13	for LDL-C ( <i>p</i> =0.03), log-TG ( <i>p</i> =0.03), and TC ( <i>p</i> =0.02) ( <b>Table 3</b> ).
14	When restricting to participants not taking lipid-lowering medication and those not taking
15	lipid- or glucose-lowering medications, respectively, the associations of GRS1 and GRS2 with
16	blood lipids were similar to those as shown above (Supplemental Table S3, S4, S5 and S6).
17	Discussion
18	To the best of our knowledge, the current study is the first Mendelian randomization
19	analysis on leptin and blood lipids. We provide robust evidence to support a potentially causal
20	relation between leptin and reduced levels of triglycerides among a majority of overweight and
21	obese population of European ancestry. Furthermore, we demonstrated that alcohol consumption
	11

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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

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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, LDLC, 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 noncurrent 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 3<sup>rd</sup>
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

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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. Acknowledgements We acknowledge editorial assistance of Ms. Jessica Ho. **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. **Funding sources** 

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

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

/ 8	Covariatos	Overall		Quartil	es of the leptir	GRS	
9	Covariates	(n=3,860)	Q1 (n=964)	Q2 (n=961)	Q3 (n=977)	Q4 (n=958)	Р
10	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)	
12	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
13	Male, N (%)	1808 (46.8)	453 (47.0)	437 (45.5)	460 (47.1)	458 (47.8)	0.89
14	Education levels, N (%)						
15 16	No more than high school	591 (15.4)	141 (14.7)	146 (15.3)	157 (16.1)	147 (15.4)	
17	Some college	1213 (31.5)	306 (31.8)	287 (30.0)	313 (32.1)	307 (32.3)	0.22
18	Bachelor's degree and above	2041 (53.1)	514 (53.5)	524 (54.8)	505 (51.8)	498 (52.3)	
19 20	Current Smoker, N (%)	603 (15.6)	144 (15.0)	152 (15.8)	165 (16.9)	142 (14.8)	0.53
20 21	Current Drinker, N (%)	3419 (89.1)	858 (89.4)	853 (89.1)	863 (88.9)	845 (89.0)	0.32
22	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
23 24	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
24 25	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
26	Treated for Lipids, N (%)	265 (6.9)	80 (8.3)	56 (5.8)	66 (6.8)	63 (6.6)	0.26
27	Treated for Diabetes, N (%)	72 (1.9)	22 (2.3)	23 (2.4)	19 (1.9)	8 (0.8)	0.03
28 29	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
30	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
31 22	BMI=body mass index; Log-leptin=logarithmically trans	sformed leptin;	GRS=Genetic	Risk Score; SI	)=standard dev	viation.	

<sup>a</sup> 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.

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

	Age, sex, BMI, waist a	djusted mod	lel b	Fully adjusted r	nodel <sup>c</sup>	b d	
	Beta(SE)	Р	-P interaction	Beta(SE)	Р	P interaction	
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.

<sup>a</sup> 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;

 <sup>b</sup> 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;

<sup>c</sup> Adjusted for age, sex, education, smoking, drinking, BMI, waist, and physical activity among the overall sample;

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

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	Age, sex, BMI, waist a	djusted model	D b	Fully adjusted	model <sup>c</sup>	D d
	Beta(SE)	Р	P interaction	Beta(SE)	Р	P interaction
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	

Table 3. Association of BMI dependent Leptin GRS2<sup>a</sup> with Baseline Lipids among Overall, Drinking, and Non-Drinking Participants of the Framingham Heart Study 3<sup>rd</sup> Generation Cohort, Respectively.

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

<sup>a</sup> 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;

<sup>b</sup> 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;

<sup>c</sup> Adjusted for age, sex, education, smoking, drinking, BMI, waist, and physical activity among the overall sample;

<sup>d</sup> 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<sup>*a*</sup> reaching genome-wide

significance (P<5×10<sup>-8</sup>)

Chromosome		Coded	Non-coded			Nearest	
Position	rsID	Allele	Allele	$\mathbf{R}^2$	Function	Gene	Population
2:27742603	rs780093	C	Т	0.995	intron variant	GCKR	European Americ
7:127860163	rs10487505	G	C	0.989	intron variant	LEP	European Americ
20:37333012	rs6071166	C	A	0.973	intergenic	SLC32A1	European Americ
BMI=body mass	index			6			
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# Supplemental Table S2. Basic Information of three SNPs for BMI dependent leptin GRS2<sup>a</sup> reaching genome-wide significance

(P<5×10<sup>-8</sup>)

	Coded	Non-coded			Nearest	
rsID	Allele	Allele	$\mathbf{R}^2$	Function	Gene	Population
rs780093	C	Т	0.995	intron variant	GCKR	European American
rs900400	Т	C	0.691	upstream variant 2KB	CCNL1	European American
rs8043757	А	CD-	0.999	intron variant	FTO	European America
lucx						
	rsID rs780093 rs900400 rs8043757	rsID     Allele       rs780093     C       rs900400     T       rs8043757     A	rsIDAlleleAllelers780093CTrs900400TCrs8043757ATndexT	rsID         Allele         Allele         R <sup>2</sup> rs780093         C         T         0.995           rs900400         T         C         0.691           rs8043757         A         T         0.999	rsIDAlleleAlleleR2Functionrs780093CT0.995intron variantrs900400TC0.691upstream variant 2KBrs8043757AT0.999intron variantndexIntegration of the second sec	rsIDAlleleAlleleR2FunctionGeners780093CT0.995intron variantGCKRrs900400TC0.691upstream variant 2KBCCNL1rs8043757AT0.999intron variantFTOndex

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

	Age, sex, BMI, waist	adjusted model	<b>D</b> b	Fully adjusted	model <sup>c</sup>	$P_{interaction}^{d}$
	Beta(SE)	Р	<i>I</i> interaction	Beta(SE)	Р	I interaction
HDL-C	Or 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	
og-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
	-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 iormeu u.<sub>b</sub>

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<sup>&</sup>lt;sup>a</sup> 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;

<sup>&</sup>lt;sup>b</sup> 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;

<sup>&</sup>lt;sup>c</sup> Adjusted for age, sex, education, smoking, drinking, BMI, waist, and physical activity among the overall sample;

<sup>&</sup>lt;sup>d</sup> Assessed by adding an interaction term, drinking×GRS to the fully adjusted model among the overall participants.

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	Age, sex, BMI, waist a	Age, sex, BMI, waist adjusted model		Fully adjusted model <sup>c</sup>		<b>D</b> . d
	Beta(SE)	Р	<i>I</i> interaction	Beta(SE)	Р	Interaction
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						

Supplemental Table S4 Association of PMI dependent Lentin CPS2<sup>a</sup> with Passing Linids among Overall Drinking and Nen

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

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<sup>&</sup>lt;sup>a</sup> 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;

<sup>&</sup>lt;sup>b</sup> 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;

<sup>&</sup>lt;sup>c</sup> Adjusted for age, sex, education, smoking, drinking, BMI, waist, and physical activity among the overall sample;

<sup>&</sup>lt;sup>d</sup> Assessed by adding an interaction term, drinking×GRS to the fully adjusted model among the overall participants.
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Supplemental Table S5. Association of BMI independent Leptin GRS1<sup>*a*</sup> with Baseline Lipids among Overall, Drinking, and Non-Drinking Participants without treating for Lipids and Diabetes of the Framingham Heart Study 3<sup>rd</sup> Generation Cohort, Respectively.

	Age, sex, BMI, waist adjusted model		$P_{interaction}^{b}$	Fully adjusted model <sup>c</sup>		$\mathbf{P}$ $d$
-	Beta(SE)	Р	I interaction	Beta(SE)	Р	
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	
.DL-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	
og-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
	-0.57 (0.28)	0.04		-0.59 (0.28)	0.04	

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Total cholesterol	
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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

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<sup>&</sup>lt;sup>*a*</sup> 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;

<sup>&</sup>lt;sup>b</sup> 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;

<sup>&</sup>lt;sup>c</sup> Adjusted for age, sex, education, smoking, drinking, BMI, waist, and physical activity among the overall sample;

<sup>&</sup>lt;sup>d</sup> 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<sup>*a*</sup> with Baseline Lipids among Overall, Drinking, and Non-Drinking Participants without treating for Lipids and Diabetes of the Framingham Heart Study 3<sup>rd</sup> Generation Cohort, respectively.

	Age, sex, BMI, waist adjusted model		<b>D</b> . b	Fully adjusted	model <sup>c</sup>	D d	
	Beta(SE)	Р	<i>r</i> interaction	Beta(SE)	Р	<i>r</i> interaction	
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		
og-TG							
Overall	-0.62 (0.23)	0.008	0.07	-0.62 (0.23)	0.008	0.00	
Not current drinkers	-2.00 (0.75)	0.008	0.06	-1.87 (0.76)	0.01	0.08	
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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

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<sup>&</sup>lt;sup>*a*</sup> 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;

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<sup>&</sup>lt;sup>c</sup> Adjusted for age, sex, education, smoking, drinking, BMI, waist, and physical activity among the overall sample;

<sup>&</sup>lt;sup>d</sup> Assessed by adding an interaction term, drinking×GRS to the fully adjusted model among the overall participants.

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

#### Heart Study the 3<sup>rd</sup> Generation cohort.

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

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

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

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

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## Reporting checklist for genetic association study.

Based on the STREGA guidelines.

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## 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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Little J, Higgins JP, Ioannidis JP, Moher D, Gagnon F, von Elm E, Khoury MJ, Cohen B, Davey-Smith related 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 to text 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.

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		Reporting Item	Number
Title	#1a	Indicate the study's design with a commonly used term in the title or the abstract	ti training, a
Abstract	#1b	Provide in the abstract an informative and balanced summary of what was done and what was found	2 2 Similar
	#2	Explain the scientific background and rationale for the investigation being reported	4 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 <sup>8</sup>
	#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 For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml	5-6

1 2 3 4 5 6 7 8 9 10 11 12	#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
13 14 15 16 17 18	#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
19 20 21	#7a	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	7
22 23 24 25 26 27	#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
28 29 30 31 32 33	#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
34 35 36 37 38 39 40 41 42 43 44 45	#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
46 47	#9a	Describe any efforts to address potential sources of bias	8
48 49 50	#9b	Describe any efforts to address potential sources of bias	8
51 52	#10	Explain how the study size was arrived at	5
53 54 55 56 57	#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.	
58 59 60	#12a	Describe all statistical methods, including those used to control for For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml	8

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	confounding. State software version used and options (or settings) chosen.
#12b	Describe any methods used to examine subgroups and interactions
#12c	Explain how missing data were addressed
#12d	If applicable, explain how loss to follow-up was addressed
#12e	Describe any sensitivity analyses
#12f	State whether Hardy-Weinberg equilibrium was considered and, if so, how.
#12g	Describe any methods used for inferring genotypes or haplotypes
#12h	Describe any methods used to assess or address population stratification.
#12i	Describe any methods used to address multiple comparisons or to control risk of false positive findings.
#12j	Describe any methods used to address and correct for relatedness among subjects
#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.
#13b	Give reasons for non-participation at each stage
#13c	Consider use of a flow diagram
#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
#14b	Indicate number of participants with missing data for each variable of interest
#14c	Cohort study – Summarize follow-up time, e.g. average and total amount.

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1 2 3 4	#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	9/10 Open: Tirst p
5 6 7 8 9 10 11 12 13 14 15		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	Protected by cop
10 17 18 19 20 21	#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	yright, includin 10-11t, includin
22 23	#16b	Report category boundaries when continuous variables were categorized	ig for c
24 25 26 27	#16c	If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	10-11 10-11 10-11
28 29	#16d	Report results of any adjustments for multiple comparisons	10-11 to
30 31 32 33	#17a	Report other analyses done—e.g., analyses of subgroups and interactions, and sensitivity analyses	t and data 10-11 data
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42 43	#18	Summarise key results with reference to study objectives	11-13sim
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study and, if applicable, for the original study on which the present article is based The STREGA checklist is distributed under the terms of the Creative Commons Attribution License n 22. ∡ in collab. CC-BY. This checklist was completed on 22. September 2018 using http://www.goodreports.org/, a tool made by the EQUATOR Network in collaboration with Penelope.ai For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml 

# **BMJ Open**

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

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

### SCHOLARONE<sup>™</sup> Manuscripts

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1 2		
3 4	1	Abstract
5 6	2	Objectives: The objective of this study was to evaluate the association of genetically determined
7 8	3	leptin with lipids.
9 10 11	4	Design: We conducted a Mendelian randomization study to assess a potential causal relationship
12 13	5	between serum leptin and lipid levels. We also evaluated whether alcohol drinking modified the
14 15	6	associations of genetically determined leptin with blood lipids.
16 17 18	7	Setting and Participants: 3,860 participants of the Framingham Heart Study 3rd Generation
19 20	8	cohort.
21 22	9	Results: Both genetic risk scores (GRSs), the GRS generated using leptin loci independent of
23 24 25	10	body mass index (BMI) and GRS generated using leptin loci dependent of BMI, were
25 26 27	11	positively associated with log transformed leptin (log-leptin). The BMI independent leptin GRS
28 29	12	was associated with log transformed triglycerides (log-TG) ( $\beta$ =-0.66, <i>p</i> =0.01), but not low
30 31	13	density lipoprotein cholesterol (LDL-C) ( $p$ =0.99), high density lipoprotein cholesterol (HDL-
32 33 34	14	C) ( $p=0.44$ ), or total cholesterol (TC) ( $p=0.49$ ). Instrumental variable estimation showed that per
35 36	15	unit increase in genetically determined log-leptin was associated with 0.55 (95% confidence
37 38	16	interval: 0.05-1.00) units decrease in log-TG. Besides significant association with log-TG ( $\beta$ =-
39 40 41	17	0.59, $p=0.009$ ), the BMI dependent GRS was nominally associated with HDL-C ( $\beta$ =-10.67,
42 43	18	$p=0.09$ ) and TC ( $\beta=-28.05$ , $p=0.08$ ). When stratified by drinking status, the BMI dependent GRS
44 45	19	was associated with reduced levels of LDL-C ( $p=0.03$ ), log-TG ( $p=0.004$ ), and TC ( $p=0.003$ )
46 47 48	20	among non-current drinkers only. Significant interactions between the BMI dependent GRS and
49 50	21	alcohol drinking were identified for LDL-C ( $p=0.03$ ), TG ( $p=0.03$ ), and TC ( $p=0.02$ ).
51 52	22	Conclusion: These findings together indicated that genetically determined leptin reduced lipid
53 54 55	23	levels and the association may be modified by alcohol consumption.
56 57 58		3

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Strengths and limitations of this study: Population-based Mendelian randomization studies may offer an opportunity to provide

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

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.

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Pleiotropy effects of SNPs included in the leptin genetic risk score (GRS) may confound • the leptin GRS and lipids associations.

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Our analyses were restricted to individuals of European ancestry.

Introduction

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2	Leptin is a key hormone that regulates appetite and food intake, body weight, and energy
3	balance. <sup>12</sup> Leptin is secreted primarily from the stomach, placenta, and adipose tissue. <sup>3</sup>
4	Biological studies have demonstrated that elevated leptin levels may play an important role in the
5	pathogenesis of lipid accumulation. <sup>4-9</sup> As an extremely active endocrine organ, the adipose tissue
6	secretes leptin playing a key role in immunometabolism. <sup>10</sup> Leptin can regulate both innate and
7	adaptive immune responses. <sup>11 12</sup> Meanwhile, leptin and insulin interact to establish a regulatory
8	feedback loop, the adipoinsular axis. <sup>13</sup> Leptin suppresses insulin synthesis and secretion from $\beta$ -
9	cells <sup>13</sup> and improves insulin sensitivity <sup>14</sup> . In turn, insulin can stimulate leptin secretion from
10	adipocytes <sup>15</sup> <sup>16</sup> . Both the immune responses and insulin are involved in lipid metabolism. <sup>17</sup> <sup>18</sup>
11	Case reports and case series have documented that leptin therapy can improve lipid profiles
12	among patients with lipoatrophy or congenital leptin deficiency. <sup>19-23</sup> On contrary, in a cross-
13	sectional survey of 12-16 years old high school students, plasma leptin was positively associated
14	with total cholesterol (TC), high density lipoprotein cholesterol (HDL-C), low-density
15	lipoprotein cholesterol (LDL-C), and triglycerides (TG). <sup>24</sup> Since observational epidemiologic
16	studies cannot rule out all confounding effects, it is unclear whether such an association is
17	causal. On the other hand, there are studies that demonstrate a neutral effect of leptin on blood
18	lipid levels. <sup>25</sup> A small clinical trial that involved 17 patients with HIV-associated lipodystrophy
19	suggested that leptin treatment did not improve fasting lipid kinetics. <sup>25</sup> Population-based
20	Mendelian randomization studies may offer an opportunity to provide better evidence for the
21	effect of leptin on lipid metabolism in the adult population. Recently, a large-scale genome-wide
22	association study (GWAS) meta-analysis identified five genomic loci associated with circulating
23	leptin, <sup>26</sup> which provides an opportunity to conduct a Mendelian randomization study to delineate

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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.<sup>27-40</sup> In rodent models, leptin has been demonstrated to be increased <sup>30-32</sup> or decreased <sup>33 34</sup> after alcohol intake. Similarly, leptin levels in human was decreased,<sup>36</sup> increased,<sup>35 37</sup> or even unchanged <sup>38-40</sup> after drinking. It is unclear whether alcohol consumption modifies the association of genetically determined leptin with lipid levels.<sup>41 42</sup> 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) 3<sup>rd</sup> 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 3<sup>rd</sup> generation cohort has been outlined in previous publications.<sup>43</sup> 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

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important covariates were available for 3,860 (94.7%) participants of the 3<sup>rd</sup> 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.<sup>26</sup> 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  $(p < 5 \times 10^{-08})$ . In addition, GCKR rs780093, CCNL1 rs900400, and FTO rs8043757 were associated with circulating leptin without adjustment for BMI.<sup>26</sup> 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.<sup>26</sup> 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,<sup>44</sup> before imputation, quality control removed SNPs with a Hardy-Weinberg equilibrium  $p < 1 \times 10^{-6}$ , 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.<sup>45 46</sup> Imputation results were summarized as 

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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  $p < 1 \times 10^{-6}$  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.<sup>47</sup> Serum leptin levels were determined by enzyme-linked immunosorbent assay (ELISA) method at R&D Systems using the Quantikine Human Leptin Immunoassay.<sup>47</sup> 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.<sup>47</sup> 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

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level weighted by their corresponding weight factor derived from the estimated oxygen
 consumption requirement for each intensity level.<sup>50</sup> 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 

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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.<sup>51</sup> 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 4.64 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<sup>2</sup> 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 diabeteslowering 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<sup>-5</sup>).

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

After controlling for age, sex, BMI, and waist circumference, log-leptin was positively associated with TC ( $\beta$ =8.56, p=6.35×10<sup>-18</sup>), LDL-C ( $\beta$ =6.46, p=1.85×10<sup>-13</sup>), and log-TG ( $\beta$ =0.13,  $p=1.59\times10^{-20}$ ), but was not associated with HDL-C ( $\beta=-0.62$ , 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 ( $\beta$ =-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 ( $\beta$ =-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 ( $\beta$ =-12.50, p=0.49) and LDL-C ( $\beta$ =-0.11, p=0.99) and positively associated with HDL-C ( $\beta=5.42$ , p=0.44), however, the correlations were not significant. The GRS1 and blood lipids associations were not modified by drinking status (Table 2). 

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

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1	As expected, the BMI dependent leptin GRS2 was not associated with any covariate
2	except for the BMI ( $p=0.02$ ) and waist circumference ( $p=0.03$ ). In the analyses controlling for
3	age, sex, BMI, and waist circumference, the GRS2 was significantly associated with lower levels
4	of log-TG ( $p$ =0.009) and nominally associated with lower levels of HDL-C ( $p$ =0.09) and TC
5	(p=0.08) (Supplemental Figure S2). When stratified by drinking status, the leptin GRS2 was
6	negatively associated with LDL-C ( $\beta$ =-92.51, p=0.03), log-TG ( $\beta$ =-2.07, p=0.004), and TC ( $\beta$ =-
7	144.68, <i>p</i> =0.003) only among non-current drinkers ( <b>Table 3</b> ). When further adjusting for
8	education, smoking, drinking, and physical activity, those associations persisted (Table 3).
9	Furthermore, significant interactions between leptin GRS2 and alcohol drinking were identified
10	for LDL-C ( <i>p</i> =0.03), log-TG ( <i>p</i> =0.03), and TC ( <i>p</i> =0.02) ( <b>Table 3</b> ).
11	When restricting to participants not taking lipid-lowering medication and those not taking
12	lipid- or glucose-lowering medications, respectively, the associations of GRS1 and GRS2 with
13	blood lipids were similar to those as shown above (Supplemental Table S3, S4, S5 and S6).
14	Discussion
14	Discussion
15	To the best of our knowledge, the current study is the first Mendelian randomization
16	analysis on leptin and blood lipids. We provide robust evidence to support a potentially causal
17	relation between leptin and reduced levels of triglycerides among a majority of overweight and
18	obese population of European ancestry. Furthermore, we demonstrated that alcohol consumption
19	modified the association of BMI dependent GRS2 with lipids in that genetically determined
20	leptin levels were inversely associated with LDL-C, log-TG, and TC, but only among individuals
21	who were not current drinkers.

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

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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 noncurrent 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.<sup>57</sup> Meanwhile, Caveolin-1 can be increased by alcohol drinking.<sup>58</sup> 

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 3<sup>rd</sup> 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.<sup>59 60</sup> 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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20 Author Contributions

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10	Data sharing statement
11	The deidentified dataset supporting this study is available on the database of genotype
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13	https://www.ncbi.nlm.nih.gov/projects/gap/cgi-bin/study.cgi?study_id=phs000007.v30.p11. The
14	researchers are able to reuse the dataset on the condition that they get the approval from dbGaP
15	and their institution.
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1 2 3 4 5 6 7 8 9	<b>Figure legends</b> <b>Figure 1.</b> The relationship between leptin, genetic risk score for leptin and triglycerides (TG) in Framingham Heart Study the 3 <sup>rd</sup> Generation cohort.
10 11 12 13 14 15 16 17 18 19 20 21 22	
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Table 1. Characteristics of the Study Participants Framingham Heart Study 3 <sup>rd</sup> Generation Cohort.	BMJ Ope	en Score 1 (GRS1	)ª for logarithn	6/bmjopen-2018-026860 or cted by copyrightAncludi iicncludi	rmed Leptin in	Page 26
	Overall		Quartile	es of the leptin (	GRS	
Covariates	(n=3,860)	Q1 (n=964)	Q2 (n=961)	(\$ <b>535</b> (\$ <b>6</b> =977)	Q4 (n=958)	Р
Genetic risk score, mean (SD)	0.07 (0.03)	0.03(0.02)	0.06 (0.01)		0.11 (0.01)	
Age, years, mean (SD)	40.2 (8.9)	40.5 (8.7)	39.9 (8.8)		39.9 (8.8)	0.23
Male, N (%)	1808 (46.8)	453 (47.0)	437 (45.5)	<b>469 (</b> 47.1)	458 (47.8)	0.89
Education levels, N (%)				t Su tex		
No more than high school	591 (15.4)	141 (14.7)	146 (15.3)	<b>1</b> <b>1</b> <b>1</b> <b>1</b> <b>1</b> <b>1</b> <b>1</b> <b>1</b> <b>1</b> <b>1</b>	147 (15.4)	
Some college	1213 (31.5)	306 (31.8)	287 (30.0)		307 (32.3)	0.22
Bachelor's degree and above	2041 (53.1)	514 (53.5)	524 (54.8)	<b><u></u><u></u><u></u><u></u><u></u><u></u><u></u><u></u><u></u><u></u><u></u><u></u><u></u><u></u><u></u><u></u><u></u><u></u><u></u></b>	498 (52.3)	
Current Smoker, N (%)	603 (15.6)	144 (15.0)	152 (15.8)	<b>16.9</b>	142 (14.8)	0.53
Current Drinker, N (%)	3419 (89.1)	858 (89.4)	853 (89.1)	<b>\$</b> 63 <b>(</b> 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)	<b>4</b> 7. <b>4</b> (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)	<b>3</b> 6. <b>8</b> (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)		36.8 (6.1)	0.70
Freated for Lipids, N (%)	265 (6.9)	80 (8.3)	56 (5.8)	$\frac{2}{6}66\frac{2}{6}6.8$	63 (6.6)	0.26
Freated for Diabetes, N (%)	72 (1.9)	22 (2.3)	23 (2.4)	<b>ဋ</b> 19 <b>ဋ</b> 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)	1 <del>1</del> .92(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 <u>4</u> 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)	92.09(65.0- 613(7.0)	90.0 (63.0- 134.0)	0.03*
Fotal Cholesterol, mg/dL, mean (SD)	188.8 (35.5)	189.1 (34.1)	188.9 (37.1)	189.5(35.7)	187.9 (35.2)	0.64
Leptin, ng/dL, median (IQR)	12.5 (3.5- 15.1)	6.7 (3.4- 14.5)	7.2 (3.4- 14.8)	7.7⊈(3.7- 1 <b>∦</b> .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 <b>°(</b> 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 <sup>6</sup> (1.0)	2.1 (1.1)	0.00005
	C 11 /	CDC C I	$\mathbf{D}^{\prime} 1 \mathbf{Q}$ $\mathbf{Q} \mathbf{D}$	1011 <sup>.</sup>		

BMI=body mass index; Log-leptin=logarithmically transformed leptin; GRS=Genetic Risk Score; SD=stand or graphique de For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml

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1 2 3 4 5 6 7 8	<sup>a</sup> Genetic risk scores 1(GRS1) for leptin was generated by summing leptin increasing alleles of 3 SNP their corresponding effect sizes reported by Kilpelainen et al. *Log transformed leptin and triglycerides were used to calculate the <i>P</i> -values.	s adjusted for BMI, weighted by s adjusted on 7 Nov
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45 46 47	For peer review only - http://binjopen.binj.com/site/about/guidelines.xittini	<u>o</u>

BMJ Open       BMJ Open         Table 2. Association of BMI independent Leptin GRS1 <sup>a</sup> with Baseline Lipids among Overall, Drinking Participants of the Framingham Heart Study 3 <sup>rd</sup> Generation Cohort, Respectively.       Solution of							
	Age, sex, BMI, waist a	djusted mod	el	Fully adjusted	nodel <sup>c</sup>		
	Beta(SE)	P	$-P_{\text{interaction}}^{\text{b}}$	Beta( SE	Р	$P_{\text{interaction}}^{d}$	
HDL-C				seig srelig			
Overall	5.42 (7.10)	0.44		7.79 (7.1 B) 19	0.27		
Not current drinkers	20.42 (18.29)	0.26	0.74	22.22 (18.76) p	0.24	0.71	
Current drinkers	6.00 (7.61)	0.43		7.02 (7.64) g š	0.36		
LDL-C				and			
Overall	-0.11 (16.09)	0.99		-1.09 (16.2) -1.09	0.95		
Not current drinkers	3.37 (48.63)	0.94	0.79	-4.18 (50.1)	0.93	0.93	
Current drinkers	-0.14 (17.10)	0.99		-1.80 (17.	0.92		
Log-TG				g, Al			
Overall	-0.66 (0.26)	0.01		-0.69 (0.2 <b>ā</b> )	0.008		
Not current drinkers	-1.41 (0.80)	0.08	0.31	-1.32 (0.83)	0.11	0.32	
Current drinkers	-0.58 (0.27)	0.04		-0.61 (0.2 <sup>9</sup>	0.03		
Total cholesterol				j.col 1d s			
Overall	-12.50 (18.21)	0.49		-12.58 (18. <b>3</b> ])	0.49		
Not current drinkers	-15.20 (54.11)	0.78	0.96	-19.13 (55. <b>6</b> 6) <b>ट</b>	0.73	0.86	
Current drinkers	-10.20 (19.37)	0.60		-11.43 (19.§2)	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.

(BMI), weighted by their corresponding effect sizes reported by Kilpelainen et al;

<sup>b</sup> Assessed by adding an interaction term, drinking×GRS along with the drinking variable to the age, sex, BM and waist adjusted model among the overall participants;

<sup>c</sup> Adjusted for age, sex, education, smoking, drinking, BMI, waist, and physical activity among the overall sagiple;

<sup>d</sup> Assessed by adding an interaction term, drinking×GRS to the fully adjusted model among the overall particepants.

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	Age, sex, BMI, waist a	adjusted model	- 1	Fully adjust		
	Beta(SE)	P	$P_{\text{interaction}}^{\circ}$	Beta(SE)	En P	- $P_{\text{interaction}}^{a}$
HDL-C				s re	seig	
Overall	-10.67 (6.20)	0.09		-10.98 (6.22) ar	<b>ne</b> 2010 0.08	
Not current drinkers	-0.69 (16.31)	0.97	0.56	0.82 (16.55) <sub>5</sub>	0.96 g	0.52
Current drinkers	-12.15 (6.64)	0.07		-11.94 (6.68) 👮	<b>S S O</b> .07	
LDL-C				tan	nloa	
Overall	-2.11 (14.05)	0.88		-2.81 (14.21) 🛱	<b>e</b> d 0.84	
Not current drinkers	-92.51 (43.02)	0.03	0.03	-101.15 (43.78) <b>ย</b> ุ้	<b>≧</b> ā 0.02	0.02
Current drinkers	9.21 (14.91)	0.54		7.89 (15.02) <b>m</b>	<b>E</b> S <b>1</b> 0.60	
log-TG				ng,	· tp	
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) A	<mark>ට</mark> 0.005	0.03
Current drinkers	-0.40 (0.24)	0.09		ق (0.24 -0.42) -0.42	0.08	
Total cholesterol				an	<u>, </u>	
Overall	-28.05 (15.91)	0.08		-28.74 (16.02) 🖻	<b>9</b> 0.07	
Not current drinkers	-144.68 (47.61)	0.003	0.02	-151.32 (48.37)	g 0.002	0.01
Current drinkers	-13.19 (16.90)	0.44		-14.67 (16.98) ธี	0.39 <b>ت</b>	

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

<sup>a</sup> Genetic risk scores2 (GRS2) for leptin was generated by summing leptin increasing alleles of 3 SNPs unadjested for body mass

index (BMI), weighted by their corresponding effect sizes reported by Kilpelainen et al;

<sup>b</sup> Assessed by adding an interaction term, drinking×GRS along with the drinking variable to the age, sex, BM and waist adjusted model among the overall participants;

<sup>c</sup> Adjusted for age, sex, education, smoking, drinking, BMI, waist, and physical activity among the overall sample;

<sup>d</sup> Assessed by adding an interaction term, drinking×GRS to the fully adjusted model among the overall partic pants.

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Position						Neārreģi	
2.27742602	rsiD	Allele	Allele	<b>R</b> <sup>2</sup>	Function	iber 201 iseig <del>ti</del> er s refete	Population
2:27742603	rs780093	С	Т	0.995	intron variant	Gorger Go	European A
7:12786016	rs10487505	G	С	0.989	intron variant	nload up®rie ∐nd	European A
20:37333012	rs6071166	С	A	0.973	intergenic	ຨ຺ຘຌ SL©SBA	European A
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Supplemental Tak (P<5×10 <sup>-8</sup> )	ble S2. Basic Ir	nformation	of three SNPs fo	or BMI dej	oendent leptin GRS2 <sup>a</sup> re:	2018-03860 on 7 No gg pyrightsincluding for	ome-wide significance
Chromosome		Coded	Non-coded			Neares	
Position	rsID	Allele	Allele	<b>R</b> <sup>2</sup>	Function	er 2019 retatien Getec	Population
2:27742603	rs780093	C	Т	0.995	intron variant	GCERP GCERP	European American
3:156798775	rs900400	Т	C	0.691	upstream variant 2KB		European American
16:53813450	rs8043757	А	T	0.999	intron variant	dard from FSCOA from m	European American
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<sup>a</sup> Genetic risk score index (BMI), weig	es 2 (GRS2) for hted by their co	leptin was g rresponding	generated by sum g effect sizes repo	ming leptin rted by Kil	n increasing alleles of 3 SI pelainen et al. m/site/about/guidelines.xhtm	e Bibling NPs unad graphique de	sted for body mass

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Supplemental Table S3. Association of BMI independent Leptin GRS1 <sup><i>a</i></sup> with Baseline Lipids among Oserall, D								
	Age, sex, BMI, waist adjusted model $P_{\text{interaction}}^{b}$ Fully adj		Age, sex, BMI, waist adjusted m		Fully adjust	<b>No</b> veodel <sup>c</sup>	P interaction	
	Beta(SE)	Р		Beta(SE)	. 2019	_		
HDL-C	-O <sub>k</sub>			to te	9. Dov			
Overall	5.25 (7.44)	0.48		7.59 (7.47) and	0.31			
Not current drinkers	18.83 (19.59)	0.34	0.86	20.43 (20.23) a 2	0.31	0.83		
Current drinkers	6.22 (7.97)	0.44		7.19 (8)	0.37			
LDL-C				Al train	/bmjop			
Overall	14.86 (16.06)	0.35		الر بو a	<b>6.</b> 41			
Not current drinkers	36.1 (49.22)	0.46	0.51	27.18 (50.69) si	0.59	0.61		
Current drinkers	12.19 (17.05)	0.47		10.48 (17.11) ar	on 0.54 נו			
log-TG				hnolog	ne 13, 2			
Overall	-0.69 (0.27)	0.01		-0.72 (0.27)	025 0.007			
Not current drinkers	-1.72 (0.84)	0.04	0.20	-1.61 (0.86)	Agen0.06	0.21		
Current drinkers	-0.58 (0.28)	0.04		-0.6 (0.28)	й Вір 0.03			
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Total cholesterol				yright, inc	2018-02680		
Overall	-0.09 (18.19)	1.00		-0.67 (18.26)	8 9 0.97		
Not current drinkers	3.78 (55.05)	0.95	0.94	-2.29 (56.42) for us n	0.97	0.97	
Current drinkers	0.98 (19.29)	0.96		-0.34 (19.32)	nber 0.99		
BMI=body mass index; GR	S=Genetic Risk Score; I	HDL-C=High-de	nsity lipoprotein	n cholesterol; LDL-C	ow-density li	ipoprotein	
				data mining, Al traini	led from http://bmjope		
				ng, and similar technologi	n.bmj.com/ on June 13, 20		
<sup>a</sup> Genetic risk scores 1 (GRS (BMI), weighted by their co <sup>b</sup> Assessed by adding an inter model among the overall par <sup>c</sup> Adjusted for age, sex, educ <sup>d</sup> Assessed by adding an inter	51) for leptin was genera rresponding effect sizes eraction term, drinking× rticipants; eation, smoking, drinkin eraction term, drinking×	tted by summing reported by Kilp GRS along with g, BMI, waist, ar GRS to the fully	leptin increasin pelainen et al; the drinking var nd physical activ adjusted model	ig alleles of 3 SNPs a iable to the age, sex, Bl wity among the overall s among the overall parti	and waist and waist and waist and ple; and waist and waist and waist	y mass index adjusted	
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	Age, sex, BMI, waist a	adjusted model	D k	Fully adjusted	<u>9</u> l model <sup>c</sup> z	D d
	Beta(SE)	Р	<b></b> <i>P</i> interaction	Beta(SE)	ovembe P	<b>P</b> interaction
HDL-C	~			relatec	er 2019	
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)	nloade 0.95	0.68
Current drinkers	-10.52 (6.94)	0.13		-10.19 (6.99)	ີຄັ້ງ 0.15	
LDL-C				iining,	n http:/	
Overall	-0.42 (14.02)	0.98		-1.56 (14.17) trai	0.91	
Not current drinkers	-83.19 (43.58)	0.06	0.07	-94.12 (44.22) ه	0.03	0.05
Current drinkers	9.71 (14.86)	0.51		7.61 (14.96) asim	0.61	
og-TG				ilar te	on Ju	
Overall	-0.63 (0.23)	0.007		-0.63 (0.23) chinolo	ne ເວິ 0.007	
Not current drinkers	-2.09 (0.74)	0.005	0.04	-2.00 (0.75)	2025 0.008 a	0.05
Current drinkers	-0.45 (0.25)	0.07		-0.47 (0.25)	Agen 0.05	
Total cholesterol					ce Bit	

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Overall	-25.28 (15.87)	0.11		-26.38 (15.97) in	-2018-0268	
Not current drinkers	-136.15 (48.44)	0.005	0.03	-144.29 (48.92)	8 9 0.003	0
Current drinkers	-10.96 (16.82)	0.51		-13.64 (16.90) u	0.42	
BMI=body mass index; GF	RS=Genetic Risk Score	; HDL-C=High-	density lipoprot	ein cholesterol; LDL-Cal	w-density lip	opro
<sup>a</sup> Genetic risk scores 2 (GR index (BMI), weighted by <sup>b</sup> Assessed by adding an int model among the overall p <sup>c</sup> Adjusted for age, sex, edu <sup>d</sup> Assessed by adding an int	S2) for leptin was gene their corresponding effect teraction term, drinking articipants; tecation, smoking, drinking teraction term, drinking	rated by summinent sizes reported wGRS along withing, BMI, waist, wGRS to the full	ng leptin increas d by Kilpelainen th the drinking v and physical ac ly adjusted mod	Al training, and similar technology, Al training, and similar technology, and	loaded from http://bmjopen.bmj.com/ on June.tag, 2005 at Agence Bibliographi	ly ma djuste
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Respectively.					1	
	Age, sex, BMI, waist a	adjusted model	P interaction <sup>b</sup>	Fully adjusted g	nodel	P i
	Beta(SE)	Р		Beta(SE) relation	P	
HDL-C	<u></u>			ed to		
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) a 5 a 5 a 5 a 5	0.27	
Current drinkers	6.22 (7.98)	0.44		7.17 (8.02)	0.37	
LDL-C				g, Altr		
Overall	15.29 (16.11)	0.34		ai. 13.42 (16.27)	0.41	
Not current drinkers	33.51 (49.77)	0.50	0.58	24.63 (51.26)	0.63	
Current drinkers	12.68 (17.09)	0.46		10.99 (17.16)	0.52	
log-TG				techno		
Overall	-0.66 (0.27)	0.01		-0.69 (0.27) gir s	<b>0.01</b>	
Not current drinkers	-1.60 (0.85)	0.06	0.23	-1.46 (0.86)	0.09	
Current drinkers	-0.57 (0.28)	0.04		-0.59 (0.28)	0.04	

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Total cholesterol					018-0268		
Overall	1.17 (18.24)	0.95		0.14 (18.33)	60 on 0.99		
Not current drinkers	6.19 (55.64)	0.91	0.93	1.04 (57.03)	Nover 0.99	0.99	
Current drinkers	1.56 (19.35)	0.94		0.26 (19.38)	nber 0.99		
BMI=body mass index; GR	S=Genetic Risk Score	; HDL-C =High	-density lipoprote	in cholesterol; LDL-C	w-density	lipoprotein	
					aded from http://bmjopen.bi rieur (ABES) . nd data mining Al training		
<sup>a</sup> Genetic risk scores 1 (GR	 S1) for leptin was gene	rated by summi	ng leptin increasi	ng alleles of 3 SNPs at	mj.com/ on June 13, 2025ted for body	v mass index	
(BMI), weighted by their co <sup>b</sup> Assessed by adding an inter model among the overall pa <sup>c</sup> Adjusted for age, sex, educe <sup>d</sup> Assessed by adding an inter	prresponding effect size eraction term, drinking articipants; cation, smoking, drinki eraction term, drinking	es reported by K ×GRS along wi ng, BMI, waist, ×GRS to the ful	A filpelainen et al; th the drinking va and physical acti lly adjusted mode	riable to the age, sex, l vity among the overall l among the overall par	BMg and waist sauple; rticipants.	adjusted	
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Su	upplemental Table S6.	Association of BMI dep	endent Leptin	GRS2 <sup>a</sup> with Bas	eline Lipids among Ove	2018-0 Ball, Drink	king, and
Dı re	rinking Participants w	ithout treating for Lipid	s and Diabetes	of the Framing	ham Heart Study 3rb G of 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5	gneration (	Cohort,
		Age, sex, BMI, waist a	djusted model		Fully adjusted	hodel <sup>c</sup>	
		Beta(SE)	Р	P interaction <sup>b</sup>	Beta(SE)	2019. P Do	P inter
H	HDL-C				ie S Xt pe	winio	
	Overall	-10.39 (6.52)	0.11		-10.48 (6.55) a (A)	aded fro	
	Not current drinkers	-8.18 (17.54)	0.64	0.95	三日 -7.77(17.87 <u>9</u> の	0.66	0.
	Current drinkers	-10.58 (6.96)	0.13		ية. -10.32 (7.01) <b>≥</b> ات	0.14	
L	LDL-C				lining	open.k	
	Overall	-1.58 (14.07)	0.91		-3.3 (14.22) and si	0.82	
	Not current drinkers	-95.04 (44.19)	0.03	0.04	-105.66 (44.8 )	g 0.02	0.
	Current drinkers	8.84 (14.90)	0.55		6.84 (15.00)	0.65	
le	og-TG				logies	2025	
	Overall	-0.62 (0.23)	0.008	0.00	-0.62 (0.23)	at 0.008	0
	Not current drinkers	-2.00 (0.75)	0.008	0.06	-1.87 (0.76)	nce 0.01 Bii	0.
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## Reporting checklist for genetic association study.

Based on the STREGA guidelines.

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

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			Page
		Reporting Item	Number
Title	#1a	Indicate the study's design with a commonly used term in the title or the abstract	Al training, a
Abstract	#1b	Provide in the abstract an informative and balanced summary of what was done and what was found	and similar 2
	#2	Explain the scientific background and rationale for the investigation being reported	technologi 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 <sup>.</sup>
	#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 For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml	5-6

1 2 3 4 5 6 7 8 9 10 11 12	#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.	
13 14 15 16 17	#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.	
19 20 21	#7a	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	
22 23 24 25 26 27	#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).	
28 29 30 31 32 33	#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.	
34 35 36 37 38 39 40 41 42 43 44	#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.	
46 47	#9a	Describe any efforts to address potential sources of bias	
48 49 50	#9b	Describe any efforts to address potential sources of bias	
51 52	#10	Explain how the study size was arrived at	
53 54 55 56 57	#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.	
58 59 60	#12a	Describe all statistical methods, including those used to control for For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml	

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	confounding. State software version used and options (or settings) chosen.
#12b	Describe any methods used to examine subgroups and interactions
#12c	Explain how missing data were addressed
#12d	If applicable, explain how loss to follow-up was addressed
#12e	Describe any sensitivity analyses
#12f	State whether Hardy-Weinberg equilibrium was considered and, if so, how.
#12g	Describe any methods used for inferring genotypes or haplotypes
#12h	Describe any methods used to assess or address population stratification.
#12i	Describe any methods used to address multiple comparisons or to control risk of false positive findings.
#12j	Describe any methods used to address and correct for relatedness among subjects
#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.
#13b	Give reasons for non-participation at each stage
#13c	Consider use of a flow diagram
#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
#14b	Indicate number of participants with missing data for each variable of interest
#14c	Cohort study – Summarize follow-up time, e.g. average and total amount.

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# **BMJ Open**

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

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

#### SCHOLARONE<sup>™</sup> Manuscripts

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1 2		
2 3 4	1	Abstract
5 6	2	<b>Objectives:</b> The objective of this study was to evaluate the association of genetically determined
7 8 9	3	leptin with lipids.
10 11	4	Design: We conducted a Mendelian randomization study to assess a potential causal relationship
12 13	5	between serum leptin and lipid levels. We also evaluated whether alcohol drinking modified the
14 15 16	6	associations of genetically determined leptin with blood lipids.
17 18	7	Setting and Participants: 3,860 participants of the Framingham Heart Study 3rd Generation
19 20	8	cohort.
21 22	9	Results: Both genetic risk scores (GRSs), the GRS generated using leptin loci independent of
23 24 25	10	body mass index (BMI) and GRS generated using leptin loci dependent of BMI, were
26 27	11	positively associated with log transformed leptin (log-leptin). The BMI independent leptin GRS
28 29	12	was associated with log transformed triglycerides (log-TG) ( $\beta$ =-0.66, <i>p</i> =0.01), but not low
30 31 32	13	density lipoprotein cholesterol (LDL-C) (p=0.99), high density lipoprotein cholesterol (HDL-
33 34	14	C) ( $p=0.44$ ), or total cholesterol (TC) ( $p=0.49$ ). Instrumental variable estimation showed that per
35 36	15	unit increase in genetically determined log-leptin was associated with 0.55 (95% confidence
37 38 20	16	interval: 0.05-1.00) units decrease in log-TG. Besides significant association with log-TG ( $\beta$ =-
40 41	17	0.59, $p=0.009$ ), the BMI dependent GRS was nominally associated with HDL-C ( $\beta$ =-10.67,
42 43	18	$p=0.09$ ) and TC ( $\beta=-28.05$ , $p=0.08$ ). When stratified by drinking status, the BMI dependent GRS
44 45	19	was associated with reduced levels of LDL-C ( $p=0.03$ ), log-TG ( $p=0.004$ ), and TC ( $p=0.003$ )
46 47 48	20	among non-current drinkers only. Significant interactions between the BMI dependent GRS and
49 50	21	alcohol drinking were identified for LDL-C ( $p=0.03$ ), TG ( $p=0.03$ ), and TC ( $p=0.02$ ).
51 52	22	Conclusion: These findings together indicated that genetically determined leptin was negatively
53 54 55	23	associated with lipid levels and the association may be modified by alcohol consumption.
50 57 58 59		3

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Strengths and limitations of this study: Population-based Mendelian randomization studies may offer an opportunity to provide

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

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.

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Pleiotropy effects of SNPs included in the leptin genetic risk score (GRS) may confound • the leptin GRS and lipids associations.

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Our analyses were restricted to individuals of European ancestry.

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#### Introduction

2 Leptin is a key hormone that regulates appetite and food intake, body weight, and energy balance.<sup>12</sup> Leptin is secreted primarily from the stomach, placenta, and adipose tissue.<sup>3</sup> 3 Biological studies have demonstrated that elevated leptin levels may play an important role in the 4 pathogenesis of lipid accumulation.<sup>4-9</sup> As an active endocrine organ, the adipose tissue secretes 5 leptin and plays a key role in immunometabolism.<sup>10</sup> Leptin can regulate both innate and adaptive 6 immune responses<sup>11 12</sup> and subsequently regulate lipid profiles. Animal study demonstrated that 7 hyperleptinemia decreases the expression of SREBP-1c, a master regulator of lipid metabolism, 8 in liver and adenovirus-induced hyperleptinemia decreases triglyceride synthesis through 9 SREBP-1c down-regulation.<sup>13</sup> Meanwhile, SREBP-1c is involved in innate immune response in 10 Macrophages<sup>14</sup>. Therefore, it is rational to see immune connects with leptin in respect of lipid 11 regulation. Case reports and case series have documented that leptin therapy can improve lipid 12 profiles among patients with lipoatrophy or congenital leptin deficiency.<sup>15-19</sup> On contrary, in a 13 14 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 15 lipoprotein cholesterol (LDL-C), and triglycerides (TG).<sup>20</sup> Since observational epidemiologic 16 studies cannot rule out all confounding effects, it is unclear whether such an association is 17 18 causal. On the other hand, there are studies that demonstrate a neutral effect of leptin on blood lipid levels.<sup>21</sup> A small clinical trial that involved 17 patients with HIV-associated lipodystrophy 19 suggested that leptin treatment did not improve fasting lipid kinetics.<sup>21</sup> Population-based 20 21 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 22 23 association study (GWAS) meta-analysis identified five genomic loci associated with circulating

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leptin, <sup>22</sup> 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. <sup>23-36</sup> In rodent models,
leptin has been demonstrated to be increased <sup>26-28</sup> or decreased <sup>29 30</sup> after alcohol intake.
Similarly, leptin levels in human was decreased, <sup>32</sup> increased, <sup>31 33</sup> or even unchanged <sup>34-36</sup> after
drinking. It is unclear whether alcohol consumption modifies the association of genetically
determined leptin with lipid levels. <sup>37 38</sup>
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) 3 <sup>rd</sup> 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 3 <sup>rd</sup> generation cohort has been outlined in previous
publications. <sup>39</sup> 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).

21 We have received approval to use the FHS data by the Institutional Review Boards at the

22 University of Georgia and the NCBI. Circulating leptin levels, genotypes, lipid levels, and

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important covariates were available for 3,860 (94.7%) participants of the 3<sup>rd</sup> 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.<sup>22</sup> 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  $(p < 5 \times 10^{-08})$ . In addition, GCKR rs780093, CCNL1 rs900400, and FTO rs8043757 were associated with circulating leptin without adjustment for BMI.<sup>22</sup> 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.<sup>22</sup> 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,<sup>40</sup> before imputation, quality control removed SNPs with a Hardy-Weinberg equilibrium  $p < 1 \times 10^{-6}$ , 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.<sup>41 42</sup> Imputation results were summarized as

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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  $p < 1 \times 10^{-6}$  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.<sup>43</sup> Serum leptin levels were determined by enzyme-linked immunosorbent assay (ELISA) method at R&D Systems using the Quantikine Human Leptin Immunoassay.<sup>43</sup> 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.<sup>43</sup> 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. 

#### 17 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

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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.<sup>46</sup> 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 logleptin 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

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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.<sup>47</sup> 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 lipidlowering 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

19 Characteristics of the study participants are presented in **Table 1**. Participants were on 20 average 40.2 years old at baseline. There were slightly more females (53.2%), and only 15.4% 21 had less than a high school education. The majority (89.1%) of the participants were current
1	drinkers, and 15.6% were current smokers. Participants were on average over weighted, with a
2	mean BMI of 26.9 kg/m <sup>2</sup> and mean waist girth of 36.6 inches. About 6.9% of the participants
3	were treated for dyslipidemia, and 1.9% were treated for diabetes. The BMI independent leptin
4	GRS1 was not associated with age ( $p=0.23$ ), sex ( $p=0.89$ ), education ( $p=0.22$ ), smoking
5	(p=0.53), drinking (p=0.32), BMI (p=0.94), waist circumference (p=0.70), lipid-lowering
6	medication usage ( $p=0.26$ ), or the physical activity index score ( $p=0.51$ ), but with diabetes-
7	lowering medication usage ( $p=0.03$ ). As expected, the GRS1 was positively associated with age,
8	sex, BMI, and waist circumference adjusted log-leptin ( $p=4.56\times10^{-5}$ ).
9	BMI independent leptin GRS1 and blood lipids
10	After controlling for age, sex, BMI, and waist circumference, log-leptin was positively
11	associated with TC ( $\beta$ =8.56, p=6.35×10 <sup>-18</sup> ), LDL-C ( $\beta$ =6.46, p=1.85×10 <sup>-13</sup> ), and log-TG ( $\beta$ =0.13,
12	$p=1.59\times10^{-20}$ ), but was not associated with HDL-C ( $\beta=-0.62$ , $p=0.11$ ) (Figure 1 and
13	Supplemental Figure S1). Per unit increase in the leptin GRS1 was associated with a 1.21-unit
14	increase in the age, sex, BMI, and waist circumference adjusted log-leptin ( $p=4.56\times10^{-5}$ ). The
15	leptin GRS1 was inversely associated with age, sex, BMI, and waist circumference adjusted log-
16	TG ( $\beta$ =-0.66, <i>p</i> =0.01) ( <b>Figure 1</b> ). When further adjusting for education, smoking, drinking, and
17	physical activity, the GRS1 and log-TG association was still significant ( $\beta$ =-0.69, p=0.008,
18	Table 2). Instrumental variable estimation indicated that log-TG levels decreased by 0.55 (95%)
19	CI: 0.05, 1.00, <i>p</i> =0.02) per unit increase of genetically determined log-leptin level ( <b>Figure 1</b> ).
20	The leptin GRS1 was inversely associated with TC ( $\beta$ =-12.50, <i>p</i> =0.49) and LDL-C ( $\beta$ =-0.11,
21	$p=0.99$ ) and positively associated with HDL-C ( $\beta=5.42$ , $p=0.44$ ), however, the correlations were

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

### 3 BMI dependent leptin GRS2 and blood lipids

4	As expected, the BMI dependent leptin GRS2 was not associated with any covariate
5	except for the BMI ( $p=0.02$ ) and waist circumference ( $p=0.03$ ). In the analyses controlling for
6	age, sex, BMI, and waist circumference, the GRS2 was significantly associated with lower levels
7	of log-TG ( $p$ =0.009) and nominally associated with lower levels of HDL-C ( $p$ =0.09) and TC
8	(p=0.08) (Supplemental Figure S2). When stratified by drinking status, the leptin GRS2 was
9	negatively associated with LDL-C ( $\beta$ =-92.51, p=0.03), log-TG ( $\beta$ =-2.07, p=0.004), and TC ( $\beta$ =-
10	144.68, <i>p</i> =0.003) only among non-current drinkers ( <b>Table 3</b> ). When further adjusting for
11	education, smoking, drinking, and physical activity, those associations persisted (Table 3).
12	Furthermore, significant interactions between leptin GRS2 and alcohol drinking were identified
13	for LDL-C ( <i>p</i> =0.03), log-TG ( <i>p</i> =0.03), and TC ( <i>p</i> =0.02) ( <b>Table 3</b> ).
14	When restricting to participants not taking lipid-lowering medication and those not taking
15	lipid- or glucose-lowering medications, respectively, the associations of GRS1 and GRS2 with
16	blood lipids were similar to those as shown above (Supplemental Table S3, S4, S5 and S6).
17	Discussion
18	To the best of our knowledge, the current study is the first Mendelian randomization
19	analysis on leptin and blood lipids. We provide robust evidence to support a potentially causal
20	relation between leptin and reduced levels of triglycerides among a majority of overweight and
21	obese population of European ancestry. Furthermore, we demonstrated that alcohol consumption
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modified association of BMI dependent GRS2 with lipids in that genetically determined leptin lev were inversely associated with LDL-C, log-TG, and TC, but only among individuals current drinkers. who were В the BMI dependent- and independent- GRSs were associated with lower level of

log-TG in current study. Inconsistent associations between leptin and blood lipids have been observed revious studies. In a small study of 80 postmenopausal women, serum leptin was ociated with HDL-C, TG, and TC, and inversely associated with LDL-C.<sup>48</sup> Another positively ted with 294 healthy school children reported that leptin was only associated with study con .<sup>49</sup> However, a study of 476 residents from Cameroon reported a positive increased correlatio etween leptin, LDL-C, and TC, and a positive association between leptin and TC, ation between leptin and HDL-C or TG.<sup>50</sup> In a more recent study of 134 physically but no as active po enopausal women, no significant correlation was detected for leptin and blood lipids.<sup>51</sup> divergent results of previous studies make it impossible to infer a relationship n and blood lipids. Possible reasons for the divergent findings include varying between failure to account for residual and unmeasured confounding, and the genetic sample s backgrou of the study population. Through Mendelian randomization analyses, we demonsti that genetically determined leptin was inversely associated with log-TG. It is well known th leles, such as risk alleles for leptin, are randomly assigned at meiosis and therefore, ent of non-genetic confounders. The association between leptin GRS and log-TG in are indep the current udy was less prone to confounding. This also highlights the importance of using Mendelia ndomization to delineate causal relationships. Our finding is further supported by previous siologic studies, among which, leptin was demonstrated to inhibit lipogenesis, plysis, and reduce triglyceride uptake.<sup>52</sup> However, the association of HDL-C and TC stimulate

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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 noncurrent 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.<sup>53</sup> Meanwhile, Caveolin-1 can be increased by alcohol drinking.<sup>54</sup> 

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 3<sup>rd</sup> 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 Page 15 of 48

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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.<sup>55 56</sup> 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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1 2 3 4 5 6 7 8 9	<b>Figure legends</b> <b>Figure 1.</b> The relationship between leptin, genetic risk score for leptin and triglycerides (TG) in Framingham Heart Study the 3 <sup>rd</sup> Generation cohort.
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Framingham Heart Study 3 <sup>rd</sup> Generation Cohort.				30 on 7 Iuding		
	Overall		Quartile	s of the leptin (	GRS	
Lovariates	(n=3,860)	Q1 (n=964)	Q2 (n=961)	( <b>§</b> 3 <b>5</b> ( <b>°</b> =977)	Q4 (n=958)	Р
Genetic risk score, mean (SD)	0.07 (0.03)	0.03(0.02)	0.06 (0.01)	Generation (0.01)	0.11 (0.01)	
Age, years, mean (SD)	40.2 (8.9)	40.5 (8.7)	39.9 (8.8)	ad 2 (9.1)	39.9 (8.8)	0.23
Male, N (%)	1808 (46.8)	453 (47.0)	437 (45.5)	<b>6 3 4 7</b> .1)	458 (47.8)	0.89
Education levels, N (%)				own text		
No more than high school	591 (15.4)	141 (14.7)	146 (15.3)	<b>a 16.1</b> )	147 (15.4)	
Some college	1213 (31.5)	306 (31.8)	287 (30.0)	រដ្ឋានុ <u>តិ</u> 32.1)	307 (32.3)	0.22
Bachelor's degree and above	2041 (53.1)	514 (53.5)	524 (54.8)	<b><u></u><u></u><u></u><u></u><u></u><u></u><u></u><u></u><u></u><u></u><u></u><u></u><u></u><u></u><u></u><u></u><u></u><u></u><u></u></b>	498 (52.3)	
Current Smoker, N (%)	603 (15.6)	144 (15.0)	152 (15.8)	<b>16.9</b>	142 (14.8)	0.53
Current Drinker, N (%)	3419 (89.1)	858 (89.4)	853 (89.1)	<b>\$</b> 63 <b>(</b> 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)	<b>A</b> 7. <b>4</b> (7.7)	37.4 (7.8)	0.51
3MI, kg/m2, mean (SD)	26.9 (5.5)	26.9 (5.5)	26.7 (5.5)	<b>3</b> 6. <b>8</b> (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)		36.8 (6.1)	0.70
Freated for Lipids, N (%)	265 (6.9)	80 (8.3)	56 (5.8)	ac (6.8)	63 (6.6)	0.26
Freated for Diabetes, N (%)	72 (1.9)	22 (2.3)	23 (2.4)	<b>s</b> 19 <b>3</b> 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)	11.92(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)	.5 <u>र्</u> च.5 <u>र्</u> च16.2)	54.4 (16.7)	0.62
Friglycerides mg/dL median (IOR)	92.0 (65.0-	92.0 (65.0-	96.0 (66.0-	₩ <u>2</u> .0¶(65.0-	90.0 (63.0-	0.03*
ringryceriaes, ing/aL, incutain (IQR)	138.0)	142.0)	140.0)	<u>o</u> 137.0)	134.0)	0.05
Γotal Cholesterol, mg/dL, mean (SD)	188.8 (35.5)	189.1 (34.1)	188.9 (37.1)	189.58 (35.7)	187.9 (35.2)	0.64
Leptin, ng/dL, median (IOR)	12.5 (3.5-	6.7 (3.4-	7.2 (3.4-	7.7 g(3.7-	7.7 (3.6-	0.02*
	15.1)	14.5)	14.8)	1 <b>8</b> .9)	16.8)	0.02
Log-repuin, mean (SD)	2.00(1.1)	1.95(1.0)	1.98 (1.1)	2.002(1.0)	2.00(1.1)	0.02
Age, sex, bivit and waist girth adjusted tog-teptin, mean (SD)	<u>2.0 (1.1)</u>	$\frac{2.0(1.0)}{CPG}$	$\frac{2.0(1.1)}{\text{Dist} \text{ Course CD}}$	$2.0_{0}(1.0)$	<u> </u>	0.00005

BMJ Open Table 1. Characteristics of the Study Participants by Genetic Risk Score 1 (GRS1)<sup>a</sup> for logarithmically gransformed Leptin in าวเ 

BMI=body mass index; Log-leptin=logarithmically transformed leptin; GRS=Genetic Risk Score; SD=stand or graphique de For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml

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1 2 3 4 5 6 7 8	<sup>a</sup> Genetic risk scores 1(GRS1) for leptin was generated by summing leptin increasing alleles of 3 SNP their corresponding effect sizes reported by Kilpelainen et al. *Log transformed leptin and triglycerides were used to calculate the <i>P</i> -values.	s adjusted for BMI, weighted by s adjusted on 7 Nov
9 10 11 12 13		ember 2019. Do Enseignement : ses related to t
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45 46 47	For peer review only - http://binjopen.binj.com/site/about/guidelines.xittini	<u>o</u>

Table 2. Association of BMI in Participants of the Framingha	BN dependent Leptin GRS1ª with m Heart Study 3 <sup>rd</sup> Generation	AJ Open Baseline Lip Cohort, Res	oids among O pectively.	6/bmjopen-2018-026860 oi cted by copyrightincludi Drinkincludi	and Non-	Drinking
	Age, sex, BMI, waist a	djusted mod	el	Fully adjusted	nodel <sup>c</sup>	
	Beta(SE)	P	$-P_{\text{interaction}}^{\text{b}}$	Beta( SE	Р	$P_{\text{interaction}}^{d}$
HDL-C				seig srelig		
Overall	5.42 (7.10)	0.44		7.79 (7.1 B) 19	0.27	
Not current drinkers	20.42 (18.29)	0.26	0.74	22.22 (18.76) p	0.24	0.71
Current drinkers	6.00 (7.61)	0.43		7.02 (7.64) g š	0.36	
LDL-C				and		
Overall	-0.11 (16.09)	0.99		-1.09 (16.2) -1.09	0.95	
Not current drinkers	3.37 (48.63)	0.94	0.79	-4.18 (50.1)	0.93	0.93
Current drinkers	-0.14 (17.10)	0.99		-1.80 (17.	0.92	
Log-TG				g, Al		
Overall	-0.66 (0.26)	0.01		-0.69 (0.2 a)	0.008	
Not current drinkers	-1.41 (0.80)	0.08	0.31	-1.32 (0.8)	0.11	0.32
Current drinkers	-0.58 (0.27)	0.04		-0.61 (0.2 <sup>9</sup>	0.03	
Total cholesterol				j.col 1d s		
Overall	-12.50 (18.21)	0.49		-12.58 (18. <b>3</b> ])	0.49	
Not current drinkers	-15.20 (54.11)	0.78	0.96	-19.13 (55. <b>6</b> 6) <b>ट</b>	0.73	0.86
Current drinkers	-10.20 (19.37)	0.60		-11.43 (19.§2)	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.

(BMI), weighted by their corresponding effect sizes reported by Kilpelainen et al;

<sup>b</sup> Assessed by adding an interaction term, drinking×GRS along with the drinking variable to the age, sex, BM and waist adjusted model among the overall participants;

<sup>c</sup> Adjusted for age, sex, education, smoking, drinking, BMI, waist, and physical activity among the overall sagiple;

<sup>d</sup> Assessed by adding an interaction term, drinking×GRS to the fully adjusted model among the overall particepants.

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	Age, sex, BMI, waist a	adjusted model	- 1	Fully adjust	l n <b>s</b> odel <sup>c</sup>	- 1
	Beta(SE)	P	$P_{\text{interaction}}^{\circ}$	Beta(SE)	En P	- $P_{\text{interaction}}^{a}$
HDL-C				s re	seig	
Overall	-10.67 (6.20)	0.09		-10.98 (6.22) ar	<b>ne</b> 2010 0.08	
Not current drinkers	-0.69 (16.31)	0.97	0.56	0.82 (16.55) <sub>5</sub>	0.96 g	0.52
Current drinkers	-12.15 (6.64)	0.07		-11.94 (6.68) 👮	<b>S S O</b> .07	
LDL-C				tan	nloa	
Overall	-2.11 (14.05)	0.88		-2.81 (14.21) 🛱	<b>e</b> d 0.84	
Not current drinkers	-92.51 (43.02)	0.03	0.03	-101.15 (43.78) <b>ย</b> ุ้	<b>≧</b> ā 0.02	0.02
Current drinkers	9.21 (14.91)	0.54		7.89 (15.02) <b>m</b>	<b>E</b> S <b>1</b> 0.60	
log-TG				ng,	· tp	
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) A	<mark>ට</mark> 0.005	0.03
Current drinkers	-0.40 (0.24)	0.09		ق (0.24 -0.42) -0.42	0.08	
Total cholesterol				an	<u>, </u>	
Overall	-28.05 (15.91)	0.08		-28.74 (16.02) 🖻	<b>9</b> 0.07	
Not current drinkers	-144.68 (47.61)	0.003	0.02	-151.32 (48.37)	g 0.002	0.01
Current drinkers	-13.19 (16.90)	0.44		-14.67 (16.98) ธี	0.39 <b>ت</b>	

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

<sup>a</sup> Genetic risk scores2 (GRS2) for leptin was generated by summing leptin increasing alleles of 3 SNPs unadjested for body mass

index (BMI), weighted by their corresponding effect sizes reported by Kilpelainen et al;

<sup>b</sup> Assessed by adding an interaction term, drinking×GRS along with the drinking variable to the age, sex, BM and waist adjusted model among the overall participants;

<sup>c</sup> Adjusted for age, sex, education, smoking, drinking, BMI, waist, and physical activity among the overall sample;

<sup>d</sup> Assessed by adding an interaction term, drinking×GRS to the fully adjusted model among the overall partic pants.

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Position						Neārreģi	
2.27742602	rsiD	Allele	Allele	<b>R</b> <sup>2</sup>	Function	iber 201 iseig <del>ti</del> er s refete	Population
2:27742603	rs780093	С	Т	0.995	intron variant	Gorger Go	European A
7:12786016	rs10487505	G	С	0.989	intron variant	nload up®rie ∐nd	European A
20:37333012	rs6071166	С	A	0.973	intergenic	ຨ຺ຘຌ SL©SBA	European A
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Supplemental Tak (P<5×10 <sup>-8</sup> )	ble S2. Basic Ir	nformation	of three SNPs fo	or BMI dej	oendent leptin GRS2 <sup>a</sup> re:	2018-03860 on 7 No gg pyrightsincluding for	ome-wide significance
Chromosome		Coded	Non-coded			Neares	
Position	rsID	Allele	Allele	<b>R</b> <sup>2</sup>	Function	er 2019 retatien Getec	Population
2:27742603	rs780093	C	Т	0.995	intron variant	GCERP GCERP	European American
3:156798775	rs900400	Т	C	0.691	upstream variant 2KB		European American
16:53813450	rs8043757	А	T	0.999	intron variant	dard from FSCOA from m	European American
						//bmjopen.bmj.com/ on June 13, 2025 at Agenc , Al training, and similar technologies.	
<sup>a</sup> Genetic risk score index (BMI), weig	es 2 (GRS2) for hted by their co	leptin was g rresponding	generated by sum g effect sizes repo	ming leptin rted by Kil	n increasing alleles of 3 SI pelainen et al. m/site/about/guidelines.xhtm	e Bibling NPs unad graphique de	sted for body mass

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Supplemental Table S3. A	Association of BMI inde	pendent Lepti Lipids of the F	n GRS1ª with Bas ramingham Heart	eline Lipids among Study 3 <sup>rd</sup> Genera	) werall, Dr g ngCohort, l	inking, and Respectivel
	Age, sex, BMI, waist	adjusted model	$P_{\text{interaction}}^{b}$	Fully adjust	<b>No</b> veodel <sup>c</sup>	P interaction
	Beta(SE)	Р		Beta(SE)	. 2019	_
HDL-C	-O <sub>k</sub>			to te	9. Dov	
Overall	5.25 (7.44)	0.48		7.59 (7.47) and	0.31	
Not current drinkers	18.83 (19.59)	0.34	0.86	20.43 (20.23) a 2	0.31	0.83
Current drinkers	6.22 (7.97)	0.44		7.19 (8)	0.37	
LDL-C				Al train	/bmjop	
Overall	14.86 (16.06)	0.35		الر بو a	<b>6.</b> 41	
Not current drinkers	36.1 (49.22)	0.46	0.51	27.18 (50.69) si	0.59	0.61
Current drinkers	12.19 (17.05)	0.47		10.48 (17.11) ar	on 0.54 נו	
log-TG				hnolog	ne 13, 2	
Overall	-0.69 (0.27)	0.01		-0.72 (0.27)	025 0.007	
Not current drinkers	-1.72 (0.84)	0.04	0.20	-1.61 (0.86)	Agen0.06	0.21
Current drinkers	-0.58 (0.28)	0.04		-0.6 (0.28)	й Вір 0.03	
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		BMJ O	lpen	cted by cop	Vbmjopen-2		Page 34 of 4
Total cholesterol				yright, inc	2018-02680		
Overall	-0.09 (18.19)	1.00		-0.67 (18.26)	8 9 0.97		
Not current drinkers	3.78 (55.05)	0.95	0.94	-2.29 (56.42) for us n	0.97	0.97	
Current drinkers	0.98 (19.29)	0.96		-0.34 (19.32)	nber 0.99		
BMI=body mass index; GR	S=Genetic Risk Score; I	HDL-C=High-de	nsity lipoprotein	n cholesterol; LDL-C	ow-density li	ipoprotein	
				data mining, Al trainir	led from http://bmjope		
				ng, and similar technologi	n.bmj.com/ on June 13, 20		
<sup>a</sup> Genetic risk scores 1 (GRS (BMI), weighted by their co <sup>b</sup> Assessed by adding an inter model among the overall pa <sup>c</sup> Adjusted for age, sex, educ <sup>d</sup> Assessed by adding an inter	51) for leptin was genera rresponding effect sizes eraction term, drinking× rticipants; eation, smoking, drinkin eraction term, drinking×	tted by summing reported by Kilf GRS along with g, BMI, waist, ar GRS to the fully	leptin increasin pelainen et al; the drinking var nd physical activ adjusted model	g alleles of 3 SNPs a iable to the age, sex, Bl vity among the overall s among the overall parti	Age and waist and waist and waist and waist and waist and waist and waist and waist and waist and waist	y mass index adjusted	
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	Age, sex, BMI, waist a	adjusted model	D k	Fully adjusted	<u>s</u> model <sup>c</sup> z	D d
	Beta(SE)	Р	<b></b> <i>P</i> interaction	Beta(SE)	ovembe	<i>P</i> interaction
HDL-C	~			relatec	er 2019	
Overall	-9.73 (6.50)	0.13		-9.66 (6.53) of the set	0.14	
Not current drinkers	-2.61 (17.44)	0.88	0.72	-1.22 (17.77) and a	nloade 0.95	0.68
Current drinkers	-10.52 (6.94)	0.13		-10.19 (6.99) a A	0.15	
LDL-C				iining,	1 http://	
Overall	-0.42 (14.02)	0.98		-1.56 (14.17) trai	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) sin	0.61	
og-TG				ilar teo	on Ju	
Overall	-0.63 (0.23)	0.007		-0.63 (0.23) chnolo	ne ເວັ 0.007	
Not current drinkers	-2.09 (0.74)	0.005	0.04	-2.00 (0.75)	2025 a	0.05
Current drinkers	-0.45 (0.25)	0.07		-0.47 (0.25)	t Agen 0.05	
Total cholesterol					ce Bit	

		BM	J Open	cted by c	3/bmjoper	
Overall	-25.28 (15.87)	0.11		-26.38 (15.97) in	2018-0268	
Not current drinkers	-136.15 (48.44)	0.005	0.03	-144.29 (48.92)	8 9 0.003	0
Current drinkers	-10.96 (16.82)	0.51		-13.64 (16.90) u	0.42	
BMI=body mass index; GF	RS=Genetic Risk Score	; HDL-C=High-	density lipoprote	ein cholesterol; LDL-C≩	w-density li	popro
<sup><i>a</i></sup> Genetic risk scores 2 (GR index (BMI), weighted by <sup><i>b</i></sup> Assessed by adding an int model among the overall p <sup><i>c</i></sup> Adjusted for age, sex, edu <sup><i>d</i></sup> Assessed by adding an int	S2) for leptin was gene their corresponding effe teraction term, drinking articipants; articipants; artation, smoking, drinking	erated by summited systems and the system of	ng leptin increas d by Kilpelainen th the drinking v and physical ac ly adjusted mode	Sing alleles of 3 SNPs upon the tal; variable to the age, sex, et and the overall s el among the overall parti	berieur (ABES) digaded from http://bmjopen.bmj.com/ on June 23 digages Ma at Agenple; icce Bibli	dy ma idjusto
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Respectively.					4	
	Age, sex, BMI, waist a	adjusted model	P interaction <sup>b</sup>		nodel	P <sub>i</sub>
	Beta(SE)	Р		Beta(SE) relation	P	_
HDL-C	<u> </u>			ed to		
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	
Current drinkers	6.22 (7.98)	0.44		7.17 (8.02)	0.37	
LDL-C				, ng, Al ti		
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	
Current drinkers	12.68 (17.09)	0.46		10.99 (17.16)	0.52	
log-TG				technu		
Overall	-0.66 (0.27)	0.01		-0.69 (0.27)	<b>3</b> 0.01	
Not current drinkers	-1.60 (0.85)	0.06	0.23	-1.46 (0.86)	0.09	
Current drinkers	-0 57 (0 28)	0.04		-0 59 (0 28)	0.04	

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		BM	U Open		6/bmjopen-2		Page 38 of 48
Total cholesterol				ŭ	.018-0268 vright, inc		
Overall	1.17 (18.24)	0.95		0.14 (18.33)	60 on 0.99		
Not current drinkers	6.19 (55.64)	0.91	0.93	1.04 (57.03)	for US 0.99	0.99	
Current drinkers	1.56 (19.35)	0.94		0.26 (19.38)	nber 0.99 nseign		
BMI=body mass index; GR	S=Genetic Risk Score	; HDL-C =High	-density lipoprote	ein cholesterol; LDL-C	w-density	lipoprotein	
					aded from http://bmjopen.br rieur (ABES) . nd data mining, Al training,		
<sup>o</sup> Genetic risk scores 1 (GRS	 S1) for leptin was gene	rated by summi	ng leptin increasi	ng alleles of 3 SNPs a	mj.com/ on June 13, 2025ed for body	v mass index	
(BMI), weighted by their co <sup>b</sup> Assessed by adding an into model among the overall pa <sup>c</sup> Adjusted for age, sex, educe	prresponding effect size eraction term, drinking articipants; cation, smoking, drinking	es reported by K GRS along wi	Kilpelainen et al; th the drinking va , and physical acti	vity among the overall	BMB and waist a	adjusted	
Assessed by adding all lift	For peer review or	nly - http://bmjope	en.bmj.com/site/abo	ut/guidelines.xhtml	rapphique de l		

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S	Supplemental Table S6.	Association of BMI dep	endent Leptin	GRS2 <sup>a</sup> with Bas	eline Lipids among Ove	2018-0 Ball, Drink	cing, and
D re	Drinking Participants with the sectively.	ithout treating for Lipid	s and Diabetes	s of the Framing	ham Heart Study 3ਾਛੂਓ ਰੂ ਯੂ <u>ਯ</u>	gneration (	Cohort,
_		Age, sex, BMI, waist ad	djusted model		Fully adjusted	anodel <sup>c</sup>	
		Beta(SE)	Р	P interaction <sup>b</sup>	Beta(SE)	2019. P Do	P inter
-	HDL-C	- Ch			Supe		
	Overall	-10.39 (6.52)	0.11		-10.48 (6.55) at (2)	aded fro	
	Not current drinkers	-8.18 (17.54)	0.64	0.95	三路 -7.77(17.87 空の	0.66	0.9
	Current drinkers	-10.58 (6.96)	0.13		-10.32 (7.01) <b>≥</b>	0.14	
	LDL-C				lining	open.k	
	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 (44.8 )	g 0.02	0.
	Current drinkers	8.84 (14.90)	0.55		6.84 (15.00)	une 0.65	
	log-TG				logies	. 2025	
	Overall	-0.62 (0.23)	0.008	0.07	-0.62 (0.23)	at 0.008	0
	Not current drinkers	-2.00 (0.75)	0.008	0.06	-1.87 (0.76)	nce 0.01 Bil	0.0
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# Reporting checklist for genetic association study.

Based on the STREGA guidelines.

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

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			Page
		Reporting Item	Number
Title	#1a	Indicate the study's design with a commonly used term in the title or the abstract	Al training, a
Abstract	#1b	Provide in the abstract an informative and balanced summary of what was done and what was found	and similar 2
	#2	Explain the scientific background and rationale for the investigation being reported	technologi 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 <sup>.</sup>
	#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 For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml	5-6

1 2 3 4 5 6 7 8 9 10 11 12	#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.	
13 14 15 16 17	#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.	
19 20 21	#7a	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	
22 23 24 25 26 27	#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).	
28 29 30 31 32 33	#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.	
34 35 36 37 38 39 40 41 42 43 44	#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.	
46 47	#9a	Describe any efforts to address potential sources of bias	
48 49 50	#9b	Describe any efforts to address potential sources of bias	
51 52	#10	Explain how the study size was arrived at	
53 54 55 56 57	#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.	
58 59 60	#12a	Describe all statistical methods, including those used to control for For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml	

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	confounding. State software version used and options (or settings) chosen.
#12b	Describe any methods used to examine subgroups and interactions
#12c	Explain how missing data were addressed
#12d	If applicable, explain how loss to follow-up was addressed
#12e	Describe any sensitivity analyses
#12f	State whether Hardy-Weinberg equilibrium was considered and, if so, how.
#12g	Describe any methods used for inferring genotypes or haplotypes
#12h	Describe any methods used to assess or address population stratification.
#12i	Describe any methods used to address multiple comparisons or to control risk of false positive findings.
#12j	Describe any methods used to address and correct for relatedness among subjects
#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.
#13b	Give reasons for non-participation at each stage
#13c	Consider use of a flow diagram
#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
#14b	Indicate number of participants with missing data for each variable of interest
#14c	Cohort study – Summarize follow-up time, e.g. average and total amount.

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1 2 3 4 5	#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	9/10 Open: Tirst p
5         6         7         8         9         10         11         12         13         14         15         16         17         18         19         20         21         22         23         24         25         26         27         28         29         30         31         32         33         34         35         36         37         38         39         40         41         42         43         44         45         46         47         48         49         50         51         52         53         54         55         56		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	ublished as 10.1136/pmjopen Protected by cop
	#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	2018-026860 on yright, includir 10-11
	#16b	Report category boundaries when continuous variables were categorized	10-111 for t
	#16c	If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	ember zun Inseignen 10-11 relater
	#16d	Report results of any adjustments for multiple comparisons	d to te 10-110 tey
	#17a	Report other analyses done—e.g., analyses of subgroups and interactions, and sensitivity analyses	t and data 10-11 data
	#17b	Report other analyses done—e.g., analyses of subgroups and interactions, and sensitivity analyses	10-111ing, Al
	#17c	Report other analyses done—e.g., analyses of subgroups and interactions, and sensitivity analyses	training, a
	#18	Summarise key results with reference to study objectives	11-13 in
	#19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias.	on June ₁3, ∠u ilar technologic 13-14
	#20	Give a cautious overall interpretation considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence.	25 at Agence в эз. 14
	#21	Discuss the generalisability (external validity) of the study results	14 Ogr
57 58	#22	Give the source of funding and the role of the funders for the present	15 15
59 60		For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml	le de l

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