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# Using Genetic Variants to Assess the Relationship Between Circulating Lipids and Type 2 Diabetes

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GENETICS/GENOMES/PROTEOMICS/METABOLOMICS

**The effects of dyslipidemia on the risk of type 2 diabetes (T2D) and related traits are not clear. We used regression models and 140 lipid-associated genetic variants to estimate associations between circulating HDL cholesterol (HDL-C), LDL cholesterol (LDL-C), and triglycerides and T2D and related traits. Each genetic test was corrected for effects of variants on the other two lipid types and surrogates of adiposity. We used the largest data sets available: 34,840 T2D case and 114,981 control subjects from the DIAGRAM (DIAbetes Genetics Replication And Meta-analysis) consortium and up to 133,010 individuals without diabetes for insulin secretion and sensitivity from the MAGIC (Meta-Analyses of Glucose and Insulin-related traits Consortium) and GENESIS (GENEticS of Insulin Sensitivity) studies. Eight of 21 associations between groups of variants and diabetes traits were significant at the nominal level, including those between genetically determined lower HDL-C ($\beta = -0.12$, $P = 0.03$) and T2D and genetically determined lower LDL-C ($\beta = -0.21$, $P = 5 \times 10^{-6}$) and T2D. Although some of these may represent causal associations, we discuss why caution must be used when using Mendelian randomization in the context of circulating lipid levels and diabetes traits. In conclusion, we found evidence of links between genetic variants associated with lipids and T2D, but deeper knowledge of the underlying genetic mechanisms of specific lipid variants is needed before drawing definite conclusions about causality based on Mendelian randomization methodology.**

Type 2 diabetes is associated with dyslipidemia (i.e., higher circulating concentrations of triglycerides and small, dense LDL cholesterol [LDL-C] and lower concentrations of HDL cholesterol [HDL-C]), but the causal relationship between dyslipidemia and type 2 diabetes has been difficult to disentangle (1). Most evidence suggests that altered lipid concentrations are secondary to insulin resistance (2) or other factors associated with both lipids and diabetes (e.g., adiposity), but some studies suggest that dyslipidemia could contribute to the pathogenesis of type 2 diabetes (3) through mechanisms related to impaired protection of β-cells or endoplasmic reticulum stress (4). Other studies of carriers of loss-of-function mutations in the *ABCA1* gene have demonstrated that altered cholesterol concentrations could affect insulin secretion in humans, although with conflicting results (5,6).

The relationship between lipid levels and diabetes is further complicated by the apparently causal link between statin therapy and increased risk of type 2 diabetes. A meta-analysis of randomized controlled trials and Mendelian randomization analysis (7) showed that statin treatment results in a slightly increased risk of diabetes. The Mendelian randomization study showed that a common allele in the 3-hydroxy-3-methylglutaryl-CoA reductase (*HMGCR*) gene (encoding the target of statins) associated with lower LDL-C was also associated with a higher risk of diabetes and that this risk is potentially mediated through a slight increase in BMI. The mechanism by which statins could increase the risk of diabetes is not known, but other theories include direct effects of statins on insulin secretion, reduced translocation of GLUT-4 to membranes in target tissues, and reduced intracellular signaling (7,8).

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Several studies have used a Mendelian randomization approach to assess the causal relationship between circulating lipids and type 2 diabetes, but these investigations included a relatively small number of studies and individuals and produced conflicting results. One study in 2,447 individuals with diabetes and 3,052 without diabetes found associations between genetically determined higher concentrations of triglycerides and lower concentrations of HDL-C and increased risk of type 2 diabetes (9). A larger study in 5,637 individuals with diabetes and up to 8,271 without diabetes using fewer genetic variants found no association between genetically determined higher concentrations of triglycerides and diabetes-related outcomes (10).

Mendelian randomization studies usually require large sample sizes because genetic variants explain only a small fraction of the primary traits under investigation. In the current study, we aimed to use genetic variants to investigate the relationships between circulating lipid fractions and type 2 diabetes by using all available data. These data included the latest genome-wide association study (GWAS) meta-analyses of 34,840 individuals with type 2 diabetes and 114,981 control subjects (11) and up to 133,010 individuals with intermediate trait measures (12,13). We assessed whether genetic variants associated with life-long differences in circulating triglyceride, HDL-C, and LDL-C levels were related to risk for type 2 diabetes and diabetes-related phenotypes, including intravenous measurements of insulin sensitivity. In this context, we also discuss the limitations of the Mendelian randomization approach when using single nucleotide polymorphisms (SNPs) that could be associated with multiple metabolic traits.

## RESEARCH DESIGN AND METHODS

### Genetic Instruments

To create genetic instruments, we selected 185 independent (linkage disequilibrium $r^2 < 0.05$) SNPs from 157 loci associated with triglycerides, HDL-C, and/or LDL-C in the most recent meta-analysis of circulating lipid concentrations in 188,577 participants (14). We started from the 185 SNPs as in Do et al. (15), who assessed the association between genetically determined lipid concentrations and coronary heart disease.

We excluded those SNPs associated at $P < 10^{-4}$ with the major confounding phenotypes BMI and waist-to-hip ratio adjusted for BMI (WHRadjBMI) (i.e., SNPs could be assumed to be primarily associated with these phenotypes) or that were associated with the outcomes (type 2 diabetes or glycemic traits) with effect sizes of >2 SDs greater than the average lipid SNP (i.e., SNPs could be assumed to be primarily associated with the outcome rather than lipid levels [see Supplementary Fig. 1 for an example and Supplementary Table 1 for a full list]). For all downstream analyses, we used a primary set of the remaining 140 SNPs as well as secondary sets of SNPs for sensitivity analyses, as follows:

1) A subset of these 140 SNPs after excluding single SNPs with disproportionately large contributions to the overall model as defined by the absolute difference of fits (DFITS) model described in the STATISTICAL ANALYSIS section;
2) Lipid-specific subsets of these 140 SNPs with relatively larger effects on the lipid fraction of interest (>0.01 SD units) and smaller effects on both the other two lipid fractions (<0.01 SD units); and
3) Subsets of SNPs identified through agglomerative hierarchical clustering as described in the STATISTICAL ANALYSIS section.

All included SNPs and their proxies are shown in Supplementary Table 2.

### Association of Lipid-Associated SNPs With Lipid Traits

We used the effect sizes of each SNP on triglycerides, HDL-C, and LDL-C from published data (14,15) wherein the per-allele effects on each lipid trait were reported in SD units based on inverse normal transformed residuals of lipid concentrations after adjustment for age and sex. For the largest cohort (deCODE genetics, $n$ = 15,612), the reported SD per lipid trait were HDL-C, 17.9 mg/dL; LDL-C, 39.9 mg/dL; and triglycerides, 81.8 mg/dL.

### Association of Lipid-Associated SNPs With Diabetes and Diabetes-Related Traits

The effect estimates of each lipid SNP on type 2 diabetes and diabetes-related traits were obtained from GWAS meta-analysis results using the largest study sizes available. The effect on type 2 diabetes risk was estimated by the DIAbetes Genetics Replication And Meta-analysis (DIAGRAM) consortium, including 34,840 case and 114,981 control subjects (11). The effect estimate of each lipid SNP on measures of glucose homeostasis was based on associations with fasting glucose in up to 133,010 participants without diabetes and 2-h glucose after an oral glucose tolerance test (OGTT) in up to 42,854 participants from the Meta-Analyses of Glucose and Insulin-related traits Consortium (MAGIC) (12). The effect of each lipid SNP on insulin sensitivity was retrieved from the following meta-analyses: *1*) insulin sensitivity based on intravenous measurement methods from the GENEticS of Insulin Sensitivity (GENESIS) consortium ($n$ = 2,764) (Supplementary Data), *2*) insulin sensitivity index (ISI) = $10{,}000/\sqrt{}$[fasting plasma glucose (mg/dL) × fasting insulin × mean glucose during OGTT (mg/dL) × mean insulin during OGTT] from an OGTT in up to 10,147 participants without diabetes from MAGIC (13), and *3*) fasting insulin (transformed to the natural logarithm scale) from up to 108,557 participants without diabetes from MAGIC (12). The SNP effects on insulin secretion were based on the OGTT-based disposition index ([(100 × insulin at 30 min)/(glucose at 30 min × [glucose at 30 min − 3.89])] × ISI) in up to 10,505 participants from MAGIC (13).

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We retrieved the effect estimates of each lipid SNP on BMI and WHRadjBMI from the Genetic Investigation of ANthropometric Traits (GIANT) consortium based on up to 249,796 individuals for BMI (16) and up to 77,167 for WHRadjBMI (17).

The alleles were aligned so that the effect allele was consistent across all phenotypes. When a SNP was not available, the proxy SNP with highest $r^2$ with the lead SNP according to HapMap2 release #24 was selected (including only proxies with $r^2 \geq 0.9$). We used phased CEU (Utah residents with Northern and Western European ancestry) haplotypes from HapMap2 release #24, build 36 (http://hapmap.ncbi.nlm.nih.gov), to identify the allele corresponding to the lipid-increasing allele reported by Do et al. (15). For some phenotypes, no information was available for lead or proxy SNPs; hence, the number of SNPs included in each analysis varied slightly.

### Statistical Analysis

#### *Primary Analysis*

For the analysis of the genetic association between circulating lipids and type 2 diabetes and diabetes-related traits, we used the framework proposed by Do et al. (15). For each outcome of interest, we used linear regression models with the SNP effect sizes on the lipid of interest ($\beta_{lipid}$) as the independent variable and the outcome of interest as the dependent variable (here, ln odds ratio for diabetes and β for related continuous traits [$\beta_{outcome}$]). If a linear relation between $\beta_{lipid}$ and $\beta_{outcome}$ is observed, causal inference can be drawn under certain assumptions, such as no population stratification, pleiotropy, or ascertainment bias. Because some lipid SNPs are associated with several lipid fractions, we ran the models with and without additional adjustment for the SNP effect on the other lipid fractions (in the same way as Do et al.) and for the SNP effects on BMI and WHRadjBMI (as some SNPs have ambiguous primary effects on lipids and these surrogate measures of adiposity). We used a Bonferroni-corrected α-threshold of 0.002 given that we analyzed seven outcome traits for three lipid fractions (0.05/21 = 0.002).

#### *Subset of Lipid SNPs Excluding Those With Disproportionately Large Contributions*

We also evaluated whether single SNPs with high influence on the primary analysis models as estimated by DFITS, as defined by Belsley et al. (18), were critical for the model results by rerunning the models without SNPs with absolute DFITS >0.4 [$2\sqrt{([k + 1]/[n - k - 1])}$], where k is the number of predictors and $n$ is the number of observations (here SNPs) (18). However, even with this approach, we note that we could not account for all sources of pleiotropy for reasons we expand on in the discussion.

#### *Subsets of SNPs With More Lipid-Specific Associations*

In sensitivity analyses, we constructed genetic risk scores for individual lipid fractions based on subsets of SNPs with relatively large effects on the lipid fraction of interest (>0.01 SD units) and smaller effects on other lipid fractions (<0.01 SD units). This approach resulted in genetic risk scores of 5, 23, and 26 SNPs for triglycerides, HDL-C, and LDL-C, respectively (Supplementary Table 2). These more-specific risk scores explained 0.2, 0.9, and 1.0% of the variance in their respective lipid levels, whereas the 140 SNPs explained 3.7, 5.8, and 6.6% for triglycerides, HDL-C, and LDL-C, respectively. Because of the small number of genetic variants and amount of phenotypic variance explained by the five triglyceride SNPs, we did not pursue this analysis any further. We then assessed the association of these scores with type 2 diabetes and diabetes-related traits on summary-level data using the method described by Dastani et al. (19) implemented in the R package gtx. Briefly, the effect of each of the three lipid scores on diabetes and related traits was calculated as Eq. 1:

$$\sum w_i \beta_i s_i^{-2} / \sum w_i^2 s_i^{-2} \qquad \text{(Eq. 1)}$$

where $\beta_i$ is the effect of the lipid-increasing alleles on diabetes, $s_i$ its corresponding SE, and $w_i$ the SNP effect on the respective lipid. We used the equation $2pq \times \beta^2$ to estimate the variance explained by the different scores, where p and q are the allele frequencies for the major and minor alleles and β the estimated effect size.

#### *Subsets of Lipid SNPs Identified Through Clustering Methods*

We used hierarchical clustering analysis to group 140 SNPs based on their effect on HDL-C, LDL-C, and triglycerides. We used Euclidean metrics to calculate pairwise distance between effect sizes on lipid levels of the 140 SNPs as input data, and we used Ward as a cluster method in the R package gplots (Supplementary Fig. 2). This method has been used before to cluster fasting insulin–associated genetic variants using metabolic traits (20). We then assessed the impact of each cluster on type 2 diabetes and related outcomes, using genetic risk scores weighted on each SNP's effect on dyslipidemia [(LDL-C + triglycerides − HDL-C)/3] and calculations using the method described by Dastani et al. (19) (see previous section on subsets of more lipid-specific SNPs).

## RESULTS

### Associations Between Genetically Determined HDL-C Levels and Diabetes Traits

#### *Primary Analysis*

We did not identify consistent associations between genetically higher circulating HDL-C and risk of type 2 diabetes as assessed by the 140-SNP set using HDL-C effect sizes for each SNP ($\beta_{HDL\text{-}C}$ vs. $\beta_{diabetes}$). In an unadjusted model, we observed a borderline association

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between genetically higher HDL-C and lower risk of type 2 diabetes ($\beta = -0.15$, $P = 2 \times 10^{-3}$), but this association was attenuated after adjusting for the effects on other lipids and surrogates of adiposity ($\beta = -0.12$, $P = 0.03$) (Table 1). Note that the coefficients can be interpreted as the estimated causal effect associated with a 1-SD unit increase in HDL-C. Hence, we estimated that a 1-SD unit increase of HDL-C was associated with an 11% reduction of relative risk of diabetes (odds ratio = $e^{-0.12} = 0.89$).

We did not identify consistent associations between genetically higher circulating HDL-C ($\beta_{HDL\text{-}C}$) and intermediate measures of glycemia in individuals without diabetes. The HDL-C ($\beta_{HDL\text{-}C}$) SNPS were associated with lower fasting glucose ($\beta_{glucose}$) in unadjusted models ($\beta = -0.04$, $P = 4 \times 10^{-5}$), but this association was attenuated when adjusting for the effects of SNPs on the other two lipid types and surrogates of adiposity ($\beta = -0.03$, $P = 3 \times 10^{-3}$). There was no association with glucose-stimulated measures of insulin secretion (disposition index). No associations were found between genetically higher circulating HDL-C and measures of insulin sensitivity by OGTT or by intravenous measurements or fasting insulin in adjusted models.

#### *Subsets of Lipid SNPs Excluding Those With Disproportionately Large Contributions*

We evaluated the impact of removing single influential SNPs by rerunning the fully adjusted models excluding the 8–13 SNPs with absolute DFITS >0.4. We found similar β-estimates, but the estimates for type 2 diabetes and fasting glucose no longer reached the Bonferroni-corrected level of significance. In this analysis, we found a positive association between HDL-C (as assessed by genetic variants) and insulin sensitivity by OGTT (Table 2).

#### *Subsets of SNPs With More Lipid-Specific Associations*

The nominal association between genetically higher circulating HDL-C and lower risk of type 2 diabetes was consistent in a sensitivity analysis of 23 SNPs with disproportionately strong associations with HDL-C compared with triglycerides and LDL-C, although the strength of significance was reduced ($\beta = -0.19$, $P = 0.04$). We also observed an association between higher HDL-C genetic risk score and lower disposition index (lower insulin secretion), which was not observed in the main analysis of 140 SNPs, but this result is inconsistent with the HDL-C risk score – type 2 diabetes association. There was no evidence of association between genetically higher circulating HDL-C and any other glycemic measures in the sensitivity analyses (Table 3).

#### *Subsets of Lipid SNPs Identified Through Clustering Methods*

We next analyzed a genetic risk score consisting of SNPs that clustered together as a group where HDL-C–lowering alleles tended to associate with neutral or positive effects on LDL-C and triglycerides (black cluster in Supplementary Fig. 2). Results from this set of SNPs were consistent with the main and other secondary analysis: The group of SNPs where alleles were associated with lower HDL-C concentrations was associated with increased risk of type 2 diabetes, raised fasting glucose levels, and lowered insulin sensitivity (Supplementary Table 3).

### Associations Between Genetically Determined LDL-C Levels and Diabetes Traits

#### *Primary Analysis*

We identified an association between genetically higher circulating LDL-C and lower risk of type 2 diabetes as assessed by the 140-SNP set using LDL-C effect sizes for each SNP ($\beta_{LDL\text{-}C}$ vs. $\beta_{diabetes}$) in the unadjusted model ($\beta = -0.14$, $P = 1 \times 10^{-3}$) and even more strongly after adjusting for effects on other lipids and surrogates of adiposity ($\beta = -0.21$, $P = 5 \times 10^{-6}$) (Table 1). We did not identify consistent associations between genetically higher circulating LDL-C ($\beta_{LDL\text{-}C}$) and intermediate glycemic traits, although there was a nominal association with lower fasting glucose in individuals without diabetes ($\beta_{LDL\text{-}C}$ vs. $\beta_{diabetes}$ [$\beta = -0.02$, $P = 0.01$]) in models adjusted for the other two lipid fractions and surrogates of adiposity. There were no associations with measures of stimulated insulin secretion or insulin sensitivity in the analyses.

#### *Subsets of Lipid SNPs Excluding Those With Disproportionately Large Contributions*

We evaluated the impact of removing single influential SNPs by rerunning the fully adjusted models excluding the 8–13 SNPs with absolute DFITS >0.4. We found similar results in this analysis as in the primary analysis (Table 2).

#### *Subsets of SNPs With More Lipid-Specific Associations*

The strength of the association of LDL-C and type 2 diabetes was dramatically smaller (and statistically nonsignificant) in a sensitivity analysis including 26 SNPs with disproportionately strong associations with LDL-C compared with triglycerides and HDL-C. There were no associations with measures of stimulated insulin secretion or insulin sensitivity in the analyses (Table 3).

#### *Subsets of Lipid SNPs Identified Through Clustering Methods*

We next analyzed a genetic risk score consisting of SNPs that clustered together as a group where LDL-C–raising alleles tended to associate with weak positive effects on triglycerides and largely neutral effects on HDL-C (red cluster in Supplementary Fig. 2). Results from this set of SNPs were consistent with other results: The group of SNPs where alleles associated with higher LDL-C was associated with a lower risk of type 2 diabetes (Supplementary Table 3). A third cluster of SNPs contained

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**Table 1—Results from linear regression of the effects of lipid-related SNPs on type 2 diabetes and diabetes-related traits**

| | Nonadjusted model | | Model adjusted for effects on other lipid fractions | | Model adjusted for effects on other lipid fractions and adiposity | | |
|---|---|---|---|---|---|---|---|
| Outcome | β (95% CI) | *P* value | β (95% CI) | *P* value | β (95% CI) | *P* value | No. SNPs in analysis |
| HDL-C | | | | | | | |
| Type 2 diabetes | −0.15 (−0.25, −0.06) | $2.2 \times 10^{-3}$ | −0.12 (−0.23, −0.01) | 0.04 | −0.12 (−0.24, −0.01) | 0.03 | 139 |
| Fasting glucose | −0.04 (−0.05, −0.02) | $4.2 \times 10^{-5}$ | −0.03 (−0.05, −0.01) | $2.6 \times 10^{-3}$ | −0.03 (−0.05, −0.01) | $3.4 \times 10^{-3}$ | 134 |
| 2-h post-OGTT glucose | −0.03 (−0.11, 0.04) | 0.40 | −0.03 (−0.12, 0.06) | 0.57 | −0.03 (−0.12, 0.06) | 0.52 | 134 |
| Insulin sensitivity* | 0.10 (−0.05, 0.25) | 0.18 | 0.12 (−0.06, 0.29) | 0.18 | 0.13 (−0.04, 0.31) | 0.15 | 140 |
| ISI from OGTT | 0.09 (0.01, 0.17) | 0.02 | 0.13 (0.03, 0.22) | 0.01 | 0.13 (0.03, 0.22) | 0.01 | 140 |
| Fasting insulin | −0.02 (−0.04, 0) | 0.01 | −0.01 (−0.03, 0.01) | 0.24 | −0.01 (−0.03, 0.01) | 0.25 | 134 |
| Disposition index | 0 (−0.08, 0.09) | 0.94 | 0.01 (−0.09, 0.1) | 0.92 | 0.01 (−0.09, 0.11) | 0.84 | 140 |
| LDL-C | | | | | | | |
| Type 2 diabetes | −0.14 (−0.23, −0.06) | $1.1 \times 10^{-3}$ | −0.20 (−0.28, −0.12) | $8.0 \times 10^{-6}$ | −0.21 (−0.29, −0.12) | $5.0 \times 10^{-6}$ | 139 |
| Fasting glucose | −0.01 (−0.03, 0) | 0.10 | −0.02 (−0.04, −0.01) | $2.9 \times 10^{-3}$ | −0.02 (−0.04, −0.01) | $5.6 \times 10^{-3}$ | 134 |
| 2-h post-OGTT glucose | 0 (−0.07, 0.07) | 0.92 | −0.01 (−0.09, 0.06) | 0.73 | −0.02 (−0.09, 0.05) | 0.59 | 134 |
| Intravenous methods (e.g., clamp) | −0.02 (−0.15, 0.12) | 0.79 | −0.01 (−0.16, 0.13) | 0.86 | 0.01 (−0.14, 0.15) | 0.93 | 140 |
| ISI from OGTT | 0 (−0.08, 0.07) | 0.91 | 0 (−0.08, 0.07) | 0.90 | 0 (−0.08, 0.08) | 0.98 | 140 |
| Fasting insulin | 0 (−0.02, 0.01) | 0.64 | −0.01 (−0.03, 0) | 0.13 | −0.01 (−0.03, 0) | 0.13 | 134 |
| Disposition index | 0.03 (−0.05, 0.11) | 0.43 | 0.03 (−0.05, 0.12) | 0.41 | 0.04 (−0.04, 0.12) | 0.31 | 140 |
| Triglycerides | | | | | | | |
| Type 2 diabetes | 0.12 (0.02, 0.23) | 0.02 | 0.13 (0.01, 0.25) | 0.03 | 0.13 (0.01, 0.25) | 0.04 | 139 |
| Fasting glucose | 0.03 (0.01, 0.05) | $2.6 \times 10^{-3}$ | 0.02 (0, 0.04) | 0.05 | 0.02 (0, 0.04) | 0.04 | 134 |
| 2-h post-OGTT glucose | 0.03 (−0.05, 0.11) | 0.50 | 0.02 (−0.08, 0.12) | 0.71 | 0.01 (−0.09, 0.11) | 0.78 | 134 |
| Intravenous methods (e.g., clamp) | −0.03 (−0.19, 0.13) | 0.71 | 0.04 (−0.16, 0.24) | 0.69 | 0.05 (−0.15, 0.24) | 0.63 | 140 |
| ISI from OGTT | 0 (−0.09, 0.08) | 0.93 | 0.07 (−0.04, 0.17) | 0.22 | 0.07 (−0.04, 0.17) | 0.21 | 140 |
| Fasting insulin | 0.03 (0.01, 0.05) | 0.01 | 0.03 (0, 0.05) | 0.03 | 0.03 (0, 0.05) | 0.03 | 134 |
| Disposition index | 0 (−0.09, 0.09) | 0.96 | −0.01 (−0.12, 0.1) | 0.88 | −0.01 (−0.12, 0.1) | 0.86 | 140 |

β (95% CI), β-coefficient with a 95% CI for association of $\beta_{lipid}$ with $\beta_{outcome}$. *Insulin sensitivity measured with euglycemic-hyperinsulinemic clamp or insulin stimulation test.



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**Table 2—Comparison of original models with models excluding SNPs with high influence as measured by DFITS**

| | Original model | | | Model excluding all SNPs with absolute DFITS >0.4 | | |
|---|---|---|---|---|---|---|
| Outcome | β (95% CI) | *P* value | No. SNPs | β (95% CI) | *P* value | No. SNPs |
| HDL-C | | | | | | |
| Type 2 diabetes | −0.12 (−0.24, −0.01) | 0.03 | 139 | −0.17 (−0.28, −0.05) | 0.01 | 128 |
| Fasting glucose | −0.03 (−0.05, −0.01) | $3.4 \times 10^{-3}$ | 134 | −0.03 (−0.05, −0.01) | 0.01 | 126 |
| 2-h post-OGTT glucose | −0.03 (−0.12, 0.06) | 0.52 | 134 | −0.05 (−0.14, 0.04) | 0.30 | 126 |
| Insulin sensitivity* | 0.13 (−0.04, 0.31) | 0.15 | 140 | 0.13 (−0.03, 0.3) | 0.12 | 132 |
| ISI from OGTT | 0.13 (0.03, 0.22) | 0.01 | 140 | 0.16 (0.07, 0.25) | $5.1 \times 10^{-4}$ | 127 |
| Fasting insulin | −0.01 (−0.03, 0.01) | 0.25 | 134 | −0.01 (−0.04, 0.01) | 0.27 | 126 |
| Disposition index | 0.01 (−0.09, 0.11) | 0.84 | 140 | −0.01 (−0.11, 0.09) | 0.83 | 132 |
| LDL-C | | | | | | |
| Type 2 diabetes | −0.21 (−0.29, −0.12) | $5.0 \times 10^{-6}$ | 139 | −0.23 (−0.32, −0.14) | $2.0 \times 10^{-6}$ | 128 |
| Fasting glucose | −0.02 (−0.04, −0.01) | $5.6 \times 10^{-3}$ | 134 | −0.02 (−0.04, −0.01) | $4.8 \times 10^{-3}$ | 126 |
| 2-h post-OGTT glucose | −0.02 (−0.09, 0.05) | 0.59 | 134 | −0.02 (−0.09, 0.05) | 0.62 | 126 |
| Insulin sensitivity* | 0.01 (−0.14, 0.15) | 0.93 | 140 | 0.03 (−0.1, 0.17) | 0.64 | 132 |
| ISI from OGTT | 0 (−0.08, 0.08) | 0.98 | 140 | 0.03 (−0.04, 0.11) | 0.40 | 127 |
| Fasting insulin | −0.01 (−0.03, 0) | 0.13 | 134 | −0.01 (−0.03, 0.01) | 0.32 | 126 |
| Disposition index | 0.04 (−0.04, 0.12) | 0.31 | 140 | 0.06 (−0.02, 0.14) | 0.16 | 132 |
| Triglycerides | | | | | | |
| Type 2 diabetes | 0.13 (0.01, 0.25) | 0.04 | 139 | 0.05 (−0.11, 0.21) | 0.53 | 128 |
| Fasting glucose | 0.02 (0, 0.04) | 0.04 | 134 | 0.02 (0, 0.04) | 0.02 | 126 |
| 2-h post-OGTT glucose | 0.01 (−0.09, 0.11) | 0.78 | 134 | −0.08 (−0.23, 0.08) | 0.33 | 126 |
| Insulin sensitivity* | 0.05 (−0.15, 0.24) | 0.63 | 140 | −0.01 (−0.28, 0.26) | 0.95 | 132 |
| ISI from OGTT | 0.07 (−0.04, 0.17) | 0.21 | 140 | 0.1 (−0.06, 0.25) | 0.22 | 127 |
| Fasting insulin | 0.03 (0, 0.05) | 0.03 | 134 | 0.02 (−0.01, 0.05) | 0.24 | 126 |
| Disposition index | −0.01 (−0.12, 0.1) | 0.86 | 140 | −0.04 (−0.14, 0.07) | 0.50 | 132 |

Models are fully adjusted for SNP effects on other lipid fractions and adiposity. β (95% CI), β-coefficient with a 95% CI for association of $\beta_{lipid}$ with $\beta_{outcome}$. *Insulin sensitivity measured with euglycemic-hyperinsulinemic clamp or insulin stimulation test.

weakly LDL-C–lowering or neutral alleles, and these alleles tended to have neutral effects on HDL-C and weak negative or neutral effects on triglycerides. This cluster of SNPs was not associated with any diabetes-related traits (Supplementary Table 3 and green cluster in Supplementary Fig. 2).

### Associations Between Genetically Determined Triglyceride Levels and Diabetes Traits

#### *Primary Analysis*

We did not identify any consistent associations between genetically higher circulating triglycerides and higher risk of type 2 diabetes as assessed by the 140-SNP set using triglyceride effect sizes for each SNP ($\beta_{triglycerides}$ vs. $\beta_{diabetes}$). We observed only very slight trends of association between genetically higher circulating triglycerides and higher risk of type 2 diabetes (β = 0.13, *P* = 0.04) and higher fasting glucose ($\beta_{glucose}$ [β = 0.02, *P* = 0.04]) and insulin ($\beta_{insulin}$ [β = 0.03, *P* = 0.03]) in individuals without diabetes in adjusted models. There were no associations with measures of stimulated insulin secretion or other measures of insulin sensitivity (Table 1).

#### *Subsets of Lipid SNPs Excluding Those With Disproportionately Large Contributions*

We evaluated the impact of removing single influential SNPs by rerunning the fully adjusted models excluding the 8–13 SNPs with absolute DFITS >0.4. In this analysis, the trends of associations were abolished for type 2 diabetes and insulin (Table 2).

## DISCUSSION

This study represents the most comprehensive genetic analysis of the role of the three main circulating lipid fractions in type 2 diabetes and related glycemic traits. We used results from the largest GWASs and 140 common genetic variants robustly associated with lipid fractions. In contrast, previous studies used a maximum of 5,637 case subjects with type 2 diabetes and 67 SNPs (9,10). The current results, therefore, add to the debate about the role of lipids in type 2 diabetes and highlight the complexities in using genetic risk scores and a Mendelian randomization approach to dissect directions of causality in complex metabolic traits and when potential pleiotropic effects or confounders exist.

Despite using data from the largest GWASs available, we did not identify consistent evidence for a causal role of circulating lipids in type 2 diabetes. We also did not identify consistent evidence for a causal role of circulating lipids with high glucose levels, insulin secretion, or insulin sensitivity measures in individuals without diabetes.

We observed 8 of 21 associations reaching a nominal $P < 0.05$. The traits studied are correlated with one another, which could explain an inflation of low *P* values. The associations were mainly observed for traits with large sample sizes (type 2 diabetes, fasting insulin, fasting glucose), and we may not have had adequate statistical

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**Table 3—Results from sensitivity analyses using subsets of lipid fraction–specific SNPs**

| Outcome | β (95% CI) | *P* value | No. SNPs |
|---|---|---|---|
| HDL-C | | | |
| Type 2 diabetes | −0.19 (−0.38, −0.01) | 0.04 | 23 |
| Fasting glucose | −0.02 (−0.06, 0.01) | 0.24 | 21 |
| 2-h post-OGTT glucose | 0.10 (−0.09, 0.28) | 0.3 | 21 |
| Insulin sensitivity* | −0.08 (−0.5, 0.34) | 0.72 | 23 |
| ISI from OGTT | 0.05 (−0.18, 0.27) | 0.69 | 23 |
| Fasting insulin | −0.02 (−0.06, 0.02) | 0.27 | 21 |
| Disposition index | −0.36 (−0.58, −0.14) | $1.0 \times 10^{-3}$ | 23 |
| LDL-C | | | |
| Type 2 diabetes | −0.03 (−0.19, 0.12) | 0.67 | 26 |
| Fasting glucose | 0 (−0.03, 0.03) | 0.85 | 23 |
| 2-h post-OGTT glucose | 0.13 (−0.04, 0.29) | 0.14 | 23 |
| Insulin sensitivity* | −0.04 (−0.41, 0.33) | 0.83 | 26 |
| ISI from OGTT | 0.08 (−0.13, 0.28) | 0.45 | 26 |
| Fasting insulin | 0.02 (−0.01, 0.06) | 0.21 | 23 |
| Disposition index | 0.05 (−0.15, 0.25) | 0.61 | 26 |

β (95% CI), β-coefficient with a 95% CI for effect of weighted lipid score on outcome. *Insulin sensitivity measured with euglycemic-hyperinsulinemic clamp or insulin stimulation test.

power for more-specific measures of insulin secretion and sensitivity. However, even the eight associations that reached nominal significance either weakened when adjusting for the effects of the SNPs on other lipids (HDL-C), weakened in sensitivity analyses (LDL-C), or could be susceptible to unaccounted-for confounding factors and biases (especially LDL-C).

We believe that the association between the HDL-C SNPs and type 2 diabetes is worthy of follow-up. The associations between HDL-C variants and type 2 diabetes and fasting glucose became weaker upon adjustment for other lipids and adiposity but remained significant when analyzing a subset of SNPs with larger effects on HDL-C. The HDL-C associations are also consistent with results from monogenic studies. There is prior genetic evidence for the causal role of HDL-C; for example, the rare mutations in the *ABCA1* gene that cause Tangier disease primarily cause low levels of circulating HDL-C and are associated with alterations in insulin secretion in humans (5,6).

The strongest association we found was that between genetically higher circulating LDL-C levels and lower risk of type 2 diabetes. This association is consistent with the recent finding of Swerdlow et al. (7) of an association between the LDL-C–lowering allele in the *HMGCR* gene (encoding the target for statins) and increased risk of type 2 diabetes. However, this association was attenuated in one of the present sensitivity analyses. We did not include the *HMGCR* variant in any of the analyses because it is associated with BMI, and this association with adiposity may explain the differences between the current results when using the more-specific set of LDL-C variants and the results of Swerdlow et al.

In some cases, we observed a discrepancy between the main analysis and the secondary analysis, most notably for the LDL-C effect on type 2 diabetes and glucose, as well as for the HDL-C effect on insulin secretion as measured by the disposition index. These discrepancies may be partly due to a relative lack of statistical power in the secondary analysis but could also be due to bias from residual pleiotropic effects.

The LDL-C-diabetes association may represent a genuine causal relationship but also provides an opportunity to discuss some of the limitations of using SNPs affecting lipids in Mendelian randomization analyses. There are several ways in which associations between lipid SNPs and diabetes could be confounded or biased. First, carrying a larger number of LDL-C–raising alleles will increase the chances of a person requiring statin therapy. Because statin therapy appears to causally increase the risk of type 2 diabetes, this could result in a noncausal association between genetically higher LDL-C and an increased risk of diabetes. However, we saw the opposite association, suggesting that such confounding was not the explanation of the findings. Second, carrying a larger number of LDL-C–raising alleles may have increased the risk of dying of a heart attack in patients with type 2 diabetes or patients too ill to participate in a study relative to survival chances in patients without diabetes. This survival bias could result in a noncausal association between genetically higher LDL-C and reduced risk of diabetes because study participants with diabetes may be depleted of LDL-C–raising alleles and could explain the current association. Future studies using longitudinal cohorts may help to solve this issue. Third, it is possible that the LDL-C effect of any lipid SNP on diabetes-related outcomes is underestimated because an LDL-C–raising SNP is more likely to result in statin therapy, which will lower LDL-C effects. Fourth, accounting for pleiotropic effects is extremely difficult. We used several approaches to minimize the influence of pleiotropy, but it is unlikely that we accounted fully for its effects, and when we did use a more LDL-C–specific

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set of SNPs, the associations with diabetes were attenuated. We had to exclude 24% of SNPs (45 of 185) with known likely pleiotropic effects, which raises the likelihood that other SNPs have unknown pleiotropic effects. Although we accounted for other lipids and surrogate measures of adiposity, including BMI and waist-to-hip ratio, we did not account fully for SNPs that may have primary effects on insulin resistance because insulin resistance is one of the main traits along a potential causal pathway (12,20). These issues have been discussed in a recent article by Burgess et al. (21) that proposed to reassess models excluding SNPs with disproportionate effects on outcome compared with their effects on lipids. We excluded SNPs with disproportionately large effects on diabetes-related outcomes, but SNPs primarily associated with insulin sensitivity may have remained in the analysis. Including only SNPs where the exact causal gene and role in lipid metabolism is not currently feasible because too little is currently known about the exact mechanisms of most SNPs, and as a result, such analyses would be underpowered. Finally, we did not have information about the effect of the included SNPs on more-specific lipid moieties and fractions, such as various free fatty acids, and we cannot exclude that the investigated SNPs have a primary effect on these moieties rather than on the investigated lipid fractions.

In conclusion, we used the largest data sets available (140 lipid SNPs, 34,840 individuals with diabetes, and up to 133,010 individuals without diabetes) and found evidence of an inverse association of both genetically determined HDL-C and LDL-C with type 2 diabetes. However, as discussed in detail, these associations could be caused by survival bias, pleiotropy, or unknown confounding factors and should be interpreted with caution.

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