Causal Graph Among Serum Lipids and Glycemic Traits: A Mendelian Randomization Study

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We systematically investigated the bidirectional causality among HDL cholesterol (HDL-C), LDL cholesterol (LDL-C), triglycerides (TGs), fasting insulin (FI), and glycated hemoglobin A_{1c} (HbA_{1c}) based on genome-wide association summary statistics of Europeans (n = 1,320,016 for lipids, 151,013 for FI, and 344,182 for HbA_{1c}). We applied multivariable Mendelian randomization (MR) to account for the correlation among different traits and constructed a causal graph with 13 significant causal effects after adjusting for multiple testing ($P < 0.0025$). Remarkably, we found that the effects of lipids on glycemic traits were through FI from TGs (β = 0.06 [95% CI 0.03, 0.08] in units of 1 SD for each trait) and HDL-C (β = −0.02 [−0.03, −0.01]). On the other hand, FI had a strong negative effect on HDL-C (β = −0.15 [−0.21, −0.09]) and positive effects on TGs (β = 0.22 [0.14, 0.31]) and HbA_{1c} (β = 0.15 [0.12, 0.19]), while HbA_{1c} could raise LDL-C (β = 0.06 [0.03, 0.08]) and TGs (β = 0.08 [0.06, 0.10]). These estimates derived from inverse-variance weighting were robust when using different MR methods. Our results suggest that elevated FI was a strong causal factor of high TGs and low HDL-C, which in turn would further increase FI. Therefore, early control of insulin resistance is critical to reduce the risk of type 2 diabetes, dyslipidemia, and cardiovascular complications.

The number of patients with diabetes aged >65 years is projected to be up to 195.2 million in 2030 and 276.2 million in 2045 globally (1). Establishing effective prevention strategies and early targeted interventions can alleviate the global health burden caused by diabetes in an aging society. Insulin resistance (IR), reflecting the compromised ability of the body to use insulin to metabolize blood glucose, occurs in the prediabetic stage and plays an important role in the development and progression of type 2 diabetes (T2D) (1,2). Identification of risk factors that are causal of IR can help in the development of effective intervention strategies for the primary prevention of T2D.

In the development of IR, fasting insulin (FI) is elevated to compensate for the reduced insulin sensitivity (3). If uncontrolled, IR can lead to β-cell dysfunction, impaired glucose tolerance, and eventually T2D. T2D is characterized by abnormally high blood glucose and is usually diagnosed when the level of glycated hemoglobin A_{1c} (HbA_{1c}) is ≥6.5%. Clinical studies have reported that higher FI is accompanied by decreased HDL cholesterol (HDL-C) and increased triglycerides (TGs) during prediabetes (3). It has been proposed to predict IR by the ratio of TGs over HDL-C in several populations (4–6). In addition, many genes associated with IR are suspected to be involved in lipid metabolism, which plays a crucial role in the development of diabetes, coronary artery disease, and other chronic diseases (7). The associations between IR and circulating HDL-C and TG levels suggest that effective management of lipid levels may facilitate prevention of IR and T2D (8–10). However, the causality of serum lipid levels in IR and T2D cannot be concluded on the basis of observational studies because of reverse causation and confounding factors.

Mendelian randomization (MR) is an instrumental variable (IV) method for causal inference between modifiable risk factors and an outcome in epidemiology (11). Using genetic variants as IVs, which are randomly assorted at meiotic segregation from parents to offspring, MR can be viewed as a "natural" randomized controlled trial to avoid the ubiquitous bias due to reverse causation and

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confounding in observational studies. Several MR studies have investigated the causality between lipid traits and IR, but their findings are inconsistent. For example, de Silva et al. (12) reported that TGs are predominantly secondary to the disease process of diabetes rather than causal, while Fall et al. (13) found that genetically higher HDL-C and TG levels are weakly associated with lower FI. A recent MR study reported no effect of lipid traits on FI but a positive effect of FI on HDL-C (14). There are relatively few MR studies between lipids and HbA_{1c}, of which conclusions are conflicting (14–17). Elevated HbA_{1c} was reported to increase HDL-C in Europeans (14) but to increase TGs and LDL-C in Chinese (15). TGs were found to have a positive effect on HbA_{1c} in one study (18) but no association in the others (14,17). These inconsistencies might be attributed to lack of statistical power given the relatively small sample sizes of early genome-wide association studies (GWAS), small numbers of IVs, or potential methodology limitations in handling horizontal pleiotropy, where IVs affect the outcome through pathways other than the exposure of interest. Furthermore, these MR studies did not account for correlations among lipid traits, hindering estimation of the direct effects of each trait, which can be disentangled by multivariable MR (MVMR) (18).

In this study, we revisited the bidirectional causal inference among serum lipids (HDL-C, LDL-C, and TGs) and the glycemic traits FI and HbA_{1c} using MR. We sought to address the aforementioned limitations by leveraging summary statistics from the largest publicly available GWAS to date and a series of state-of-the-art MR methods. First, we performed MVMR to infer causal relationships among three lipid traits. Second, we examined the bidirectional causality between each lipid trait and glycemic trait by univariable MR (UVMR). Third, considering the genetic and phenotypic correlation among lipid traits, we estimated independent effects of lipid traits on each glycemic trait using MVMR (19). Finally, we estimated causal effects between FI and HbA_{1c}, adjusting for lipid traits in MVMR. Taken together, we constructed a causal graph between lipid and glycemic traits.

RESEARCH DESIGN AND METHODS

GWAS Summary Statistics

We downloaded GWAS summary statistics for lipid traits, HbA_{1c}, and FI (Supplementary Table 1) from the Global Lipids Genetics Consortium (GLGC) (20), UK Biobank (UKB) (21), and Meta-Analyses of Glucose and Insulin-related traits Consortium (MAGIC) (22). For lipids, we collected three sets of GWAS summary statistics: GLGC analysis of 1,320,016 Europeans (20), GLGC analysis of 930,672 Europeans without UKB participants (20), and UKB analysis of 361,194 White British participants by the Neale Laboratory at the Broad Institute (21). We obtained GWAS summary statistics of HbA_{1c} from UKB (n = 344,182) (21) and of FI with adjustment of BMI from MAGIC (n = 151,013 Europeans without diabetes) (22). We noted that the MAGIC samples were included in the GLGC analysis (20), leading to sample overlap between two data sets (Fig. 1*A*). To avoid bias due to sample overlap, we designed two-sample MR analyses using different data sets for different exposure-outcome pairs, as illustrated in Fig. 1*B* and detailed in Supplementary Table 2.

To facilitate interpretation of effect estimates, we calculated the mean and SD of lipid traits and HbA_{1c} with individual-level data from UKB (n = 472,671 White participants) (Supplementary Table 2). We assumed that the SDs of lipid traits in GLGC were similar to those in UKB because GLGC did not provide SDs of its lipid traits. For FI, the MAGIC study reported sample size, mean, and SD from each contributing cohort (22). Because the MAGIC study used log-transformed FI (log-FI) in GWAS, we calculated the mean and SD of log-FI for each contributing cohort by assuming a log-normal distribution of FI and then averaged across cohorts weighted by the sample size (Supplementary Table 3). We confirmed that single nucleotide polymorphism (SNP) effect sizes in GWAS summary statistics were all reported on the basis of the normalized traits, except for FI from MAGIC, which was based on log-FI without normalization (22). We thus standardized SNP effect sizes (and the corresponding SEs) from MAGIC by dividing the estimated SD of log-FI.

SNP Heritability and Genetic Correlation

We calculated the SNP heritability (h^2_{SNP}) and genetic correlation (r_g) using the linkage disequilibrium (LD) score regression method (23). In LD score regression analyses, we used summary statistics from GLGC for lipid traits (n = 1,320,016, including UKB samples), MAGIC for FI (n = 151,013), and UKB for HbA_{1c} (n = 344,182). The LD score of each SNP was calculated on the basis of the 1000 Genomes Project phase 3 European samples (24). Following the user manual, LD score regression analyses were based on autosomal SNPs in HapMap 3, excluding the MHC region (25).

Selection of IVs

The IV selection procedure is illustrated in Fig. 1*C*. We first selected candidate IVs for each exposure-outcome pair. SNPs with GWAS sample sizes <50,000 were excluded to minimize bias from estimating errors. We started with SNPs in significant association with the exposure ($P < 5 \times 10^{-8}$) and present in the summary data of both the exposure and the outcome. We excluded SNPs that failed the Steiger directionality test (one-sided $P < 0.05$) (26) and SNPs in LD ($r^2 > 0.01$ within 10 Mb based on 1000 Genomes Project Europeans) (27). To minimize horizontal pleiotropy, we further searched candidate IVs in PhenoScanner version 2 (28) and excluded those associated ($P < 5 \times 10^{-8}$) with blood pressure, BMI, C-reactive protein, hematological indices, and renal and hepatic function indices (Supplementary Table 4).

For MVMR, we started with a union set of the candidate IVs for all exposure-outcome pairs. We then excluded SNPs associated ($P < 5 \times 10^{-8}$) with the other lipid or

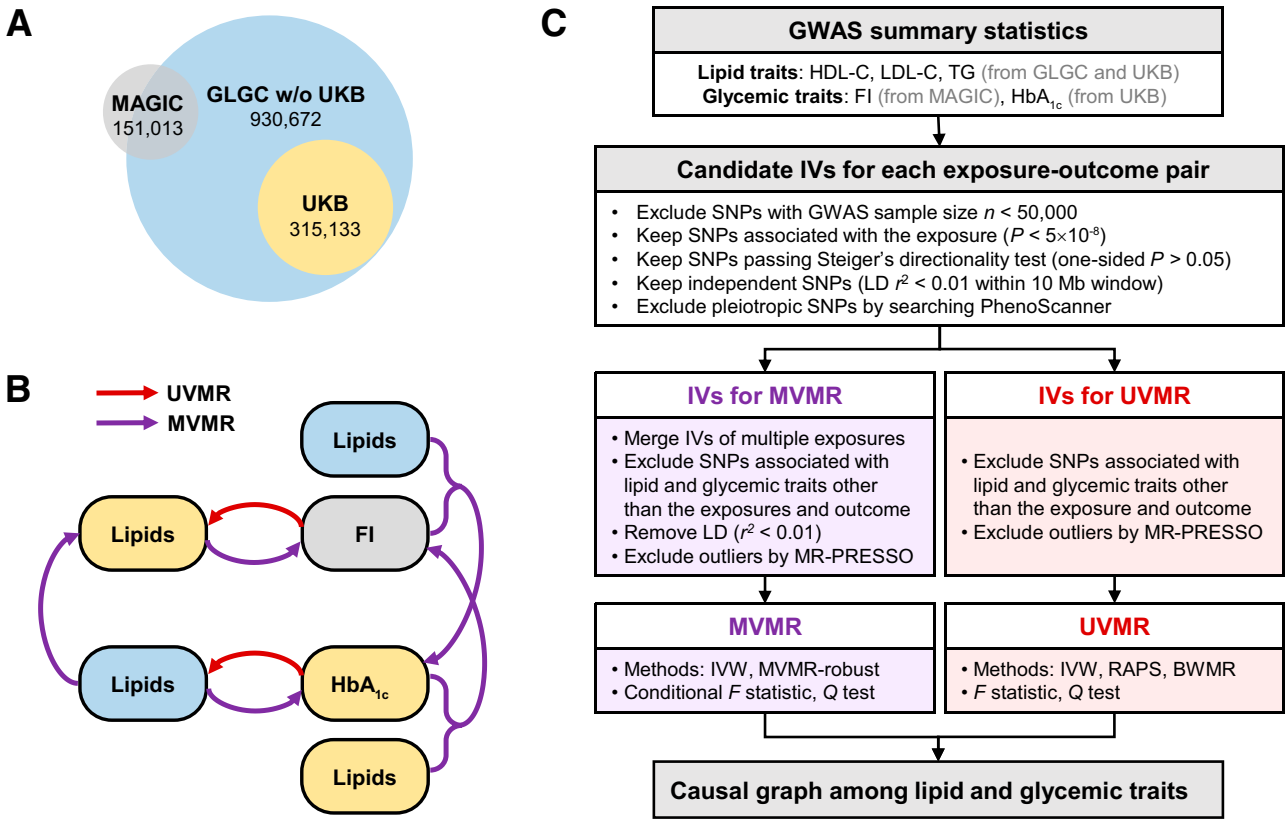


Figure 1—Study design for the MR analysis. *A*: Sample overlap of data sets used in the analyses. GLGC w/o UKB indicates GLGC without UKB samples. The area of each circle and the number reflect the sample size. *B*: MR analyses in this study. Each arrow represents one MR analysis or one set of MR analyses, pointing from the exposure(s) to the outcome(s). Box colors indicate data sets shown in *A*. *C*: Flowchart of IV selection and MR analysis.

glycemic traits (except for the exposures and outcome) in our GWAS summary statistics or in LD (r^2 >0.01) with other candidate IVs. Finally, we applied the MR Pleiotropy Residual Sum and Outlier (MR-PRESSO) method to detect and remove horizontal pleiotropic SNPs ($P < 0.05$) (29). For UVMR, we excluded candidate IVs associated ($P < 5 \times 10^{-8}$) with the other lipid or glycemic traits (except for the exposure and outcome) in our GWAS summary statistics and applied MR-PRESSO to remove outliers ($P < 0.05$). The final IVs are listed in Supplementary Tables 5–21.

MR Analyses

For MVMR, we applied two methods: inverse-variance weighting (IVW) (19) and MVMR-robust. The IVW method is unbiased given no pleiotropy (19). The MVMR-robust method is robust to pleiotropic SNPs by using a robust regression technique to attenuate the influence of outlier IVs. For UVMR, we used three methods: IVW under a multiplicative random effects model (30), Bayesian-weighted MR (BWMR) (31), and robust adjusted profile score (RAPS) (32). Both BWMR and RAPS are proposed to handle the weak instrumental bias and horizontal pleiotropy. For both MVMR and UVMR, we reported the IVW estimates as the main results and used the other methods as sensitivity analyses.

We calculated *F* and *Q* statistics to confirm robustness of our MR estimates (33). We reported the standard *F* statistic for UVMR and the conditional *F* statistic for MVMR. $F < 10$ indicates potential weak instrumental bias (34). We calculated the *Q* statistic for heterogeneity tests in both UVMR and MVMR. A statistically significant *Q* statistic suggests the presence of invalid IVs. To estimate the conditional *F* statistic and *Q* statistic for MVMR, we assumed phenotypic correlations as those estimated from individual-level data of UKB (n = 472,671 White participants) (Supplementary Table 22) if summary statistics of exposures were from the same sample and no correlations if exposures were from different samples (33).

MR analyses were performed using the packages MVMR (33), BWMR (31), and TwoSampleMR version 0.4.25 in R version 3.6.3. Because we tested bidirectional causal effects among five traits, we defined statistical significance as $P < 0.0025$ (0.05/20) to account for multiple testing of 20 exposure-outcome pairs.

Data and Resource Availability

GWAS summary statistics were downloaded from public websites (UKB, https://pan.ukbb.broadinstitute.org; GLGC,

https://csg.sph.umich.edu/willer/public/glgc-lipids2021; MAGIC, https://magicinvestigators.org). Individual-level phenotype data of UKB were accessed under application no. 63454 (resources available upon request from https://www.ukbiobank.ac.uk).

RESULTS

Genetic Correlations Among Lipid and Glycemic Traits

HDL-C, LDL-C, TGs, FI, and HbA_{1c} were estimated to have SNP heritability of 0.122 (95% CI 0.100, 0.146), 0.081 (0.064, 0.99), 0.095 (0.080, 0.111), 0.096 (0.079, 0.113), and 0.182 (0.158, 0.206), respectively (Fig. 2 and Supplementary Table 23). FI had a strong negative genetic correlation with HDL-C (r_g = −0.657 [95% CI −0.722, −0.593]; $P = 6.85 \times 10^{-88}$) and a positive genetic correlation with TGs (r_g = 0.380 [0.287, 0.473]; $P = 8.94 \times 10^{-16}$). In contrast, no significant genetic correlation between FI and LDL-C was observed, despite strong correlations among LDL-C, HDL-C, and TGs. HbA_{1c}, on the other hand, was genetically correlated with all lipid traits and FI (HDL-C: r_g = −0.175 [−0.226, −0.124; $P = 2.48 \times 10^{-11}$]; LDL-C: r_g = 0.183 [0.129, 0.236; $P = 2.10 \times 10^{-11}$]; TGs: r_g = 0.296 [0.228, 0.364; $P = 1.25 \times 10^{-7}$]; FI: r_g = 0.127 [0.049, 0.204; P = 0.001]). The high genetic correlation among lipid and glycemic traits justifies the choice of MVMR to infer their causal relationships.

Causal Relationships Among Lipids

We performed MVMR analyses, treating one lipid trait as the outcome and the other two as the exposures (Fig. 3). All conditional F statistics were large, suggesting no weak instrumental bias. HDL-C and TGs showed negative causal effects on each other (HDL-C on TG: β_{IVW} = −0.11 [95% CI −0.13, −0.08; $P = 3.31 \times 10^{-16}$]; TG on HDL-C: β_{IVW} = −0.20 [−0.23, −0.18; $P = 1.50 \times 10^{-48}$]), while LDL-C and TGs showed bidirectional positive effects (LDL-C on TG: β_{IVW} = 0.04 [0.02, 0.06; $P = 3.20 \times 10^{-4}$]; TGs on LDL-C: β_{IVW} = 0.19 [0.17, 0.21; $P = 3.03 \times 10^{-62}$]). In addition, HDL-C had a positive effect on LDL-C (β_{IVW} = 0.06 [0.04, 0.08]; $P = 5.48 \times 10^{-12}$) independent of TGs. Because all traits have been normalized, the estimated effect sizes should be interpreted in the units of 1 SD of each trait, which are 0.38 mmol/L for HDL-C, 0.87 mmol/L for LDL-C, and 0.52 log(mmol/L) for log-TGs on the basis of data from UKB (Supplementary Table 1).

Causal Relationships Between Lipids and FI

We performed MVMR to estimate causal effects of three lipid traits on FI (Fig. 4*A*). We identified 660 IVs for lipids in the MVMR analysis with conditional F statistics >10 for all exposures. We found that decreased HDL-C (β_{IVW} = −0.02 [95% CI −0.03, −0.01]; P = 0.002) and elevated TGs (β_{IVW} = 0.06 [0.03, 0.08]; $P = 3.23 \times 10^{-7}$) would lead to higher FI but no significant causal effect of LDL-C on FI (P = 0.379). The effect sizes should be interpreted in the unit of 1 SD of the natural log-FI, which was 0.63 log(pmol/L) (Supplementary Table 1).

In the reverse direction, we performed UVMR to estimate causal effects of FI on each lipid trait, in which seven IVs were identified for FI (Fig. 4*B*). Despite a small number of IVs, the F statistic was 36.5, suggesting no weak instrumental bias. We found strong causal effects of FI on HDL-C (β_{IVW} = −0.21 [95% CI −0.33, −0.08]; P = 0.001) and TGs (β_{IVW} = 0.20 [0.11, 0.28]; $P = 6.55 \times 10^{-6}$) but not on LDL-C (P = 0.095). Estimates from the RAPS and BWMR methods were almost identical to those from IVW (Supplementary Table 25).

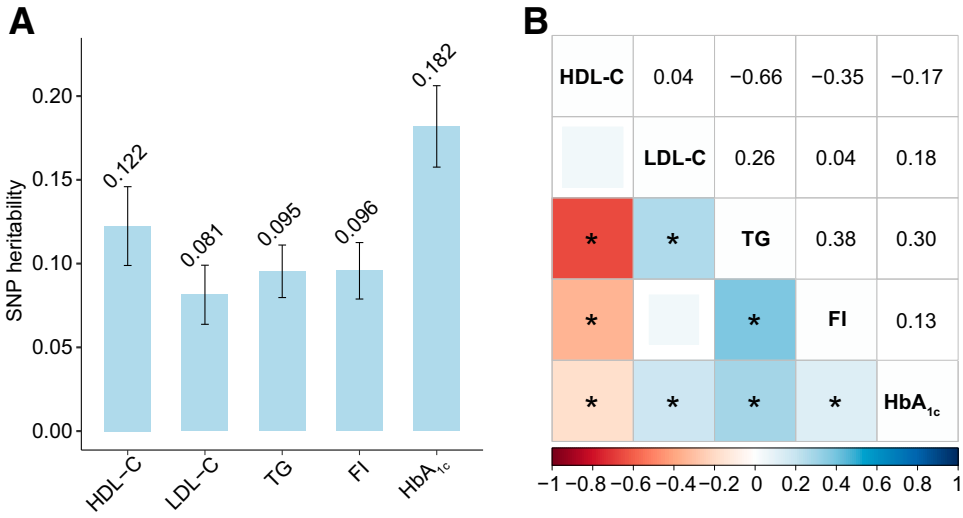


Figure 2—Genetic relationships among HDL-C, LDL-C, TGs, FI, and HbA_{1c}. *A*: SNP heritability of each trait. The error bars indicate the 95% CI, and the numbers above are point estimates. *B*: Genetic correlations among HDL-C, LDL-C, TGs (from GLGC), FI (from MAGIC), and HbA_{1c} (from UKB). Numbers show the estimated values. Solid squares represent $P < 0.05$. *$P < 0.0025$ for genetic correlations.

Exposure	Outcome	No. IVs	*F*	Beta (95% CI)	*P*
LDL−C	HDL−C	710	91.8	−0.02 (−0.04, 0.00)	0.053
TG			44.4	−0.20 (−0.23, −0.18)	**1.50×10^{-48}**
HDL−C	LDL−C	948	80.5	0.06 (0.04, 0.08)	**5.48×10^{-12}**
TG			51.0	0.19 (0.17, 0.21)	**3.03×10^{-62}**
HDL−C	TG	820	69.4	−0.11 (−0.13, −0.08)	**3.31×10^{-16}**
LDL−C			79.4	0.04 (0.02, 0.06)	**3.20×10^{-4}**

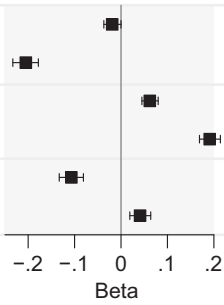


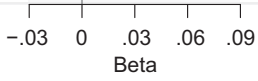
Figure 3—MVMR analyses among lipids. Shown are the number of IVs and the conditional *F* statistic for each exposure in MVMR. Significance at $P < 0.0025$ is highlighted in boldface type.

We also performed MVMR analyses to estimate the causal effect of FI on HDL-C (or TGs), adjusting for TGs (or HDL-C) (Fig. 4*B*). The effect of FI on HDL-C was attenuated after adjusting for TGs but remained highly significant with more IVs in MVMR ($\beta_{IVW} = -0.15$ [95% CI −0.21, −0.09]; $P = 8.47 \times 10^{-7}$; $\beta_{MVMR\text{-}robust} = -0.16$ [−0.23, −0.09]; $P = 4.76 \times 10^{-6}$). For TGs, the estimated effect of FI after adjusting for HDL-C was similar to that derived from UVMR using the IVW method ($\beta_{IVW} = 0.22$ [0.14, 0.31]; $P = 3.59 \times 10^{-7}$), whereas MVMR-robust yielded a slightly smaller, but consistent estimate ($\beta_{MVMR\text{-}robust} = 0.13$ [0.04, 0.23]; $P = 0.005$). These results confirm that FI has an independent causal effect on HDL-C and TGs.

While we detected no heterogeneity in the UVMR analyses of FI on lipids, *Q* statistics were significant in all MVMR analyses, suggesting that horizontal pleiotropy might have not been completely removed despite our stringent IV selection criteria. Furthermore, the conditional *F* statistics were <10 for FI in the MVMR analyses because most IVs were associated with TGs or HDL-C rather than FI. Reassuringly, causal effect estimates of FI from both the UVMR analyses and the MVMR-robust method were largely consistent with

A

Exposure	Outcome	No. IVs	*F*	Beta (95% CI)	*P*
HDL−C	FI	660	103.1	−0.02 (−0.03, −0.01)	**0.002**
LDL−C			39.2	−0.01 (−0.03, 0.01)	0.379
TG			50.9	0.06 (0.03, 0.08)	**3.23×10^{-7}**



B

Exposure	Outcome	No. IVs	*F*	Beta (95% CI)	*P*
FI	HDL−C	7	36.5	−0.21 (−0.33, −0.08)	**0.001**
FI	LDL−C	7	36.5	0.08 (−0.01, 0.16)	0.095
FI	TG	7	36.5	0.20 (0.11, 0.28)	**6.55×10^{-6}**
FI	HDL−C	256	5.7	−0.15 (−0.21, −0.09)	**8.47×10^{-7}**
TG			63.6	−0.25 (−0.28, −0.21)	**3.24×10^{-48}**
FI	TG	358	3.5	0.22 (0.14, 0.31)	**3.59×10^{-7}**
HDL−C			39.7	−0.12 (−0.15, −0.08)	**8.89×10^{-13}**

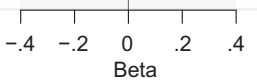


Figure 4—Bidirectional MR analyses between lipids and FI. *A*: MVMR analysis of lipids on FI. *B*: UVMR and MVMR analyses of FI on lipids. Effect of FI on HDL-C (or TGs) was estimated using MVMR adjusting for TGs (or HDL-C). Shown are the number of IVs in each analysis and the conditional *F* statistic for each exposure in MVMR and standard *F* statistic in UVMR. Significance at $P < 0.0025$ is highlighted in boldface type.

the IVW estimates in MVMR, supporting the validity of our results (Supplementary Table 25).

Causal Relationships Between Lipids and HbA_{1c}

We next investigated the causal relationships between lipids and HbA_{1c}. We estimated from individual-level data of UKB that the SD of HbA_{1c} was 0.59% (or 6.52 mmol/mol) (Supplementary Table 1). In the MVMR analyses of lipid traits on HbA_{1c}, we detected no significant effects, despite a large number of IVs with large conditional *F* statistics (Fig. 5*A*). In the reverse direction, we found positive effects of HbA_{1c} on LDL-C (β_{IVW} = 0.06 [95% CI 0.04, 0.07]; $P = 6.71 \times 10^{-13}$) and TGs (β_{IVW} = 0.06 [0.04, 0.08]; $P = 6.35 \times 10^{-13}$) in UVMR analyses (Fig. 5*B*). Furthermore, the effect of HbA_{1c} on LDL-C was largely unchanged after adjusting for TGs in MVMR (β_{IVW} = 0.06 [0.03, 0.08]; $P = 6.10 \times 10^{-7}$), and similarly on TGs after adjusting for LDL-C (β_{IVW} = 0.080 [0.06, 0.10]; $P = 1.28 \times 10^{-11}$), confirming independent effects of HbA_{1c} on LDL-C and TGs. Despite significant heterogeneity detected by *Q* tests, BWMR and RAPS methods in the UVMR analyses and MVMR-robust method in the MVMR analyses yielded similar results as the IVW method (Supplementary Table 26).

Causal Relationships Between Glycemic Traits

Conditioning on the lipid traits, MVMR analyses suggested a strong positive effect of FI on HbA_{1c} (β_{IVW} = 0.15 [95% CI 0.12, 0.19]; $P = 8.97 \times 10^{-22}$) and a negative effect for HbA_{1c} on FI ($\beta_{IVW} = -0.04$ [−0.06, −0.02]; $P = 5.58 \times 10^{-4}$) (Fig. 6). Estimates from the MVMR-robust method were similar, but the effect of HbA_{1c} on FI only reached nominal significance, despite a consistent effect size ($\beta_{MVMR\text{-}robust} = -0.04$ [−0.07, −0.01]; P = 0.011) (Supplementary Table 27). Furthermore, we observed that the negative effect of HDL-C on FI and positive effect of TGs on FI remained significant after conditioning on HbA_{1c} (Fig. 6). Null results for lipids on HbA_{1c} were also replicated when conditioning on FI. Most conditional *F* statistics were >10 except for that of FI, which was only 3.0 because of the small number of IVs associated with FI. Nevertheless, the effect of FI on HbA_{1c} estimated by MVMR-robust was similar to that from IVW ($\beta_{MVMR\text{-}robust}$ = 0.13 [0.09, 0.17]; $P = 3.36 \times 10^{-21}$) (Supplementary Table 27).

DISCUSSION

We systematically investigated the bidirectional causal relationships among lipid traits (HDL-C, LDL-C, and TGs) and glycemic traits (FI and HbA_{1c}) using MR analyses based on summary statistics from the largest GWAS to date. Taking all the MR estimates together, we constructed a causal graph with 13 significant causal links (Fig. 7). Because all GWAS were based on the normalized traits, our estimated effect sizes were in the same unit of 1 SD of each trait,

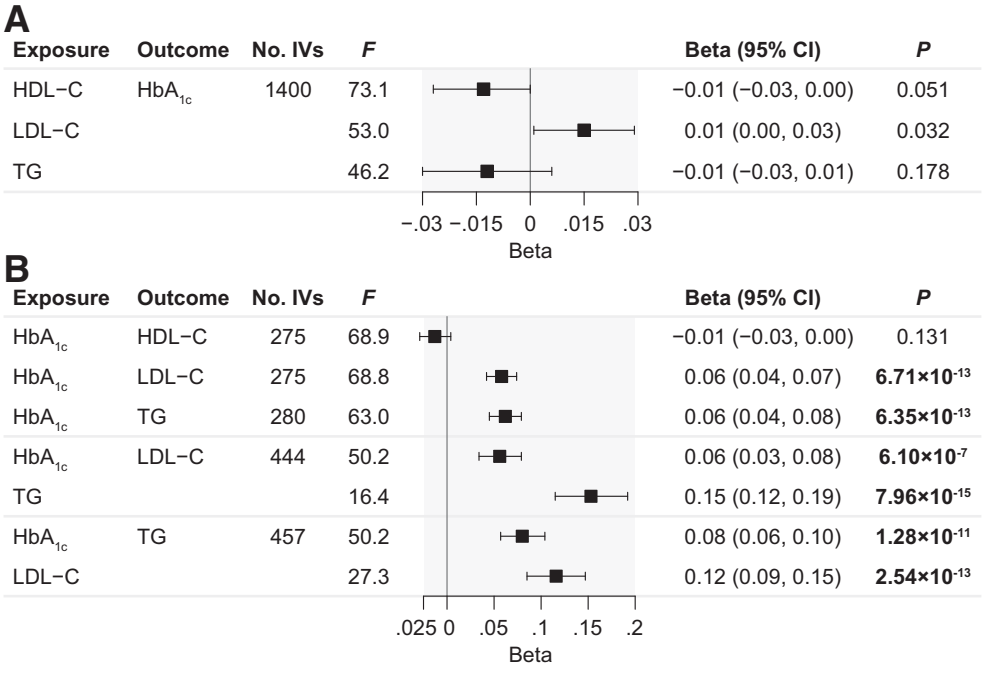


Figure 5—Bidirectional MR analyses between lipids and HbA_{1c}. *A*: MVMR analysis of lipids on HbA_{1c}. *B*: UVMR and MVMR analyses of HbA_{1c} on lipids. Effect of HbA_{1c} on LDL-C (or TGs) was estimated using MVMR adjusting for TGs (or LDL-C). Shown are the number of IVs in each analysis and the conditional *F* statistic for each exposure in MVMR and standard *F* statistic in UVMR. Significance at $P < 0.0025$ is highlighted in boldface type.

Exposure	Outcome	No. IVs	*F*	Beta (95% CI)	*P*
HbA_{1c}	FI	971	26.0	−0.04 (−0.06, −0.02)	**5.58×10^{-4}**
HDL−C			48.0	−0.02 (−0.04, −0.01)	**4.06×10^{-4}**
LDL−C			26.3	−0.01 (−0.03, 0.01)	0.560
TG			25.5	0.06 (0.04, 0.08)	**3.99×10^{-8}**
FI	HbA_{1c}	1383	3.0	0.15 (0.12, 0.19)	**8.97×10^{-22}**
HDL−C			27.4	−0.01 (−0.03, 0.00)	0.157
LDL−C			50.7	0.02 (0.01, 0.03)	0.008
TG			28.4	−0.02 (−0.04, 0.00)	0.039

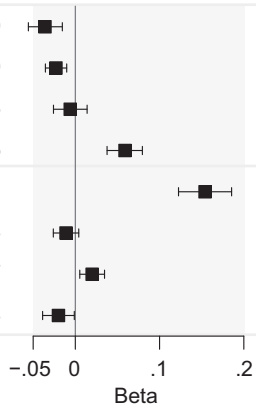


Figure 6—Bidirectional MVMR analyses between FI and HbA_{1c}, adjusting for lipids. Shown are the number of IVs and the conditional *F* statistic for each exposure in MVMR. Significance at $P < 0.0025$ is highlighted in boldface type.

enabling comparison of effect sizes across traits. We found that TGs show significant bidirectional causal effects with LDL-C, HDL-C, and FI, while HDL-C and LDL-C had weaker or no effect on glycemic traits. FI had strong causal effects on TG, HDL-C, and HbA_{1c}, while HbA_{1c} had moderate causal effects on LDL-C and TGs, but not on HDL-C. These results featured TGs and FI as key biomarkers and potential intervention targets in the progression of IR, T2D, and cardiovascular complications.

As mentioned in the *Introduction*, previous MR studies have reported inconsistent results on the relationships among HDL-C, TGs, and FI based on small numbers of IVs and GWAS of small sample sizes (12–14,35). In contrast, our findings are more reliable because we used summary statistics from the largest GWAS to date, adopted strict criteria to select valid IVs, and applied several MR methods that are robust to pleiotropy. Furthermore, our findings of the causal roles of HDL-C and TGs on FI are supported by several biological studies. For example, TGs are implicated in the pathogenesis of IR by inducing plasma free fatty acids, which can reduce insulin receptor tyrosine kinase activity, destabilize the insulin receptor, and reduce insulin-stimulated glycogen synthase activity (36). HDL-C has been reported to have a positive effect on β-cell survival and insulin secretion through cholesterol homeostasis, and suppression of inducible nitric oxide synthase and fatty acid synthase were reported (3,37).

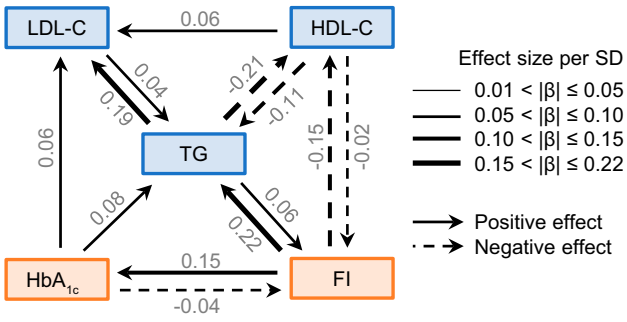


Figure 7—Causal graph among lipids, FI, and HbA_{1c}. Significant causal effects ($P < 0.0025$) are presented as arrows with the estimated effect sizes alongside. All traits have been standardized so that the effect sizes are comparable in units (SD/SD).

The strong causal effects of FI on HDL-C and TG are consistent with the hypothesis that IR can elevate VLDL and TGs in the liver either directly or through lipolysis to induce free fatty acids in adipose tissue (8). Furthermore, IR might lower HDL-C by increasing cholesterol ester conversion from HDL-C to the VLDL-TG complex (3,38) or by increasing hepatic TG lipase activity to accelerate clearance of HDL in the kidney (36). Consistent with the progression from IR to T2D, we found that elevated FI had a strong effect to cause accumulation of HbA_{1c}, while high HbA_{1c} could result in reduced FI. HbA_{1c}, on the other hand, had moderate positive effects on LDL-C and TGs, which are consistent with the risk effect of T2D on cardiovascular diseases but have not been identified by previous MR studies. The causal effects of FI and HbA_{1c} on lipids imply that complications of T2D, such as dyslipidemia and cardiovascular diseases, might have been initiated at the prediabetes stage when FI is elevated, highlighting the importance of early control of IR.

It is well known that statin treatment to lower plasma lipids can increase the risk of T2D. The intended drug target of statins is HMG-CoA reductase. Inhibition of HMG-CoA reductase can impair hepatocyte cholesterol synthesis, increase hepatic LDL receptor expression, and thus reduce circulating LDL-C. Both genetic analysis and clinical trials support a causal role of HMG-CoA reductase inhibition on the elevated risk of T2D and body weight gain (39). A recent genetic study further suggested that statin treatment can increase HbA_{1c} and lower sex hormone

binding globulin in females, and these pleiotropic effects do not seem to be mediated by LDL-C (40). An alternative mechanistic explanation of the association between statin treatment and T2D is mediation through body weight gain. Our MVMR analysis identified a very weak causal effect of LDL-C on HbA_{1c} at a nominal significance level (β_{IVW} = 0.01 [95% CI 0.00, 0.03]; P = 0.032), suggesting that LDL-C is unlikely the mediator between statin treatment and higher T2D risk.

Our study has several limitations. First, like in many MR studies, it is difficult to confirm no bias caused by horizontal pleiotropy, especially when a large number of IVs is needed to achieve sufficient statistical power. To mitigate this issue, we developed stringent IV selection criteria and adopted several MR methods that were robust to horizontal pleiotropy. Second, we did not consider other important indices of IR, such as HOMA-IR and HOMA-B, because their GWAS sample sizes were relatively small (the largest sample size was 51,750 from the MAGIC study [41]). Third, the GWAS data sets of FI and HbA_{1c} used in our MR analyses were based on samples mostly without diabetes. Therefore, caution and further investigation are needed when extrapolating our causal effect estimates to patients with T2D with abnormally high FI or HbA_{1c}. Fourth, our estimated effect sizes were in the unit of 1 SD per trait, but the values of SDs were not available for all GWAS data sets. Instead, we estimated SDs for lipids and HbA_{1c} based on phenotype data of UKB White participants and assumed that GLGC samples had the same variation. Furthermore, we estimated the SD of log-FI by assuming that FI followed a log-normal distribution in each contributing cohort of MAGIC. Inaccurate estimation of SDs might mislead the interpretation of the magnitudes of causal effects but would not affect the statistical significance. Finally, the current study is based on samples of European ancestry. While the genetic architecture of complex diseases has been found to be largely shared among different populations, further replication in non-European populations will ensure general transferability of our findings.

In conclusion, our systematic MR analyses elucidate complex relationships among lipid and glycemic traits and provide profound insights into the disease progression of IR, T2D, and cardiovascular complications. Our results suggest that effective management of TGs and HDL-C might help to reduce FI and the progression of IR, and the risk effect of T2D on dyslipidemia and cardiovascular disease might start from the prediabetic stage, highlighting the importance of timely control of IR in the prevention of T2D, dyslipidemia, and cardiovascular complications.

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