Roles of Cardiometabolic Factors in Mediating the Causal Effect of Type 2 Diabetes on Cardiovascular Diseases: A Two-Step, Two-Sample Multivariable Mendelian Randomization Study

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Abstract

Objectives

To investigate the roles of cardiometabolic factors (including blood pressure, blood lipids, thyroid function, body mass, and insulin sensitivity) in mediating the causal effect of type 2 diabetes (T2DM) on cardiovascular disease (CVD) outcomes.

Design

Two-step, two-sample multivariable Mendelian randomization (MVMR) study.

Setting

International genome-wide association study (GWAS) consortia data.

Exposure

T2DM, blood pressure: systolic blood pressure (SBP), diastolic blood pressure (DBP); blood lipids: low-density lipoprotein (LDL), high-density lipoprotein (HDL), total cholesterol (TC), triglycerides (TG); thyroid function: hyperthyroidism, hypothyroidism; body mass index (BMI), waist-hip-ratio (WHR), and insulin sensitivity.

Main outcomes

CVD including coronary heart disease (CHD), myocardial infarction (MI) and stroke.

Methods

Summary-level data for exposures and main outcomes were extracted from GWAS consortia. We used two-sample MR to illustrate the causal effect of T2DM on CVD subtypes and regression-based MVMR to quantify the possible mediation effects of cardiometabolic factors on CVD.

Results

Each additional unit of log odds of T2DM increased 16% risk of CHD [OR: 1.16, 95% confidence interval (CI): 1.12-1.21], 15% risk of MI (OR: 1.15, 95%CI: 1.10-1.20), and 10% risk of stroke (OR: 1.10, 95%CI: 1.06-1.13). In mediation analysis, SBP, DBP and TG were found as main mediators, while the mediation effects of other cardiometabolic factors were not significant. The proportion of total effect of T2DM on CHD mediated by SBP, DBP and TG was 16% (95%CI: 8%-24%), 7% (95%CI: 1%-13%) and 10% (95%CI: 2%-18%), respectively. Mediation effect of SBP and DBP on MI and stroke, TG on MI was also prominent, while mediation effect of TG on stroke was not significant. Combined mediation effect of all three mediators accounted for 29%, 26% and 13% of total effect of T2DM on CHD, MI and stroke, respectively.

Conclusion

SBP, DBP and TG mediate a substantial proportion of the causal effect of T2DM on CVD and thus interventions on these factors might reduce considerable excess risk of CVD among T2DM patients.

1. Introduction

Globally, cardiovascular disease (CVD) remains the leading cause of mortality which accounts for over 17 million deaths annually (1). Compelling observational studies have proved that type 2 diabetes (T2DM), which will influence up to 550 million patients by 2030 (2), has always been supposed to be associated with increased risk of CVD (3, 4). It is estimated that T2DM patients over 50 years old lost 6 years in average than non-diabetic population, and 58% of this difference can be attributable to CVD (5). However, controversial evidence from previous studies showed that intensive glycemic control per se might even increase CVD risk (6). Thus, exploring treatment strategies besides from glycemic control is essential for management of T2DM patients. As patients with T2DM are often present with multiple cardiometabolic disorders, understanding whether these risk factors have roles in mediating the causal effect of T2DM on CVD would provide new intervention targets to reduce excess CVD risk for T2DM patients.

An observational study has investigated the influence of T2DM on subtype of CVD mediated by blood pressure, cholesterol, glucose and other metabolic factors individually and concluded that decreasing systolic blood pressure (SBP) and total cholesterol (TC)/ high-density lipoprotein (HDL) ratio could reduce 10-year CVD risk (7). Besides, an open, parallel trial aiming at T2DM patients with mean follow-up of 7.8 years concluded that intensified interventions including decline glycosylated hemoglobin, blood pressure, TC, triglycerides (TG) and urinary albumin excretion rate brought huge benefit in reducing CVD events by 50% (8). However, observational studies have always been criticized for their weakness in proving causal associations because of unknown or inadequately measured confounding factors. Moreover, conducting a well-designed randomized-controlled trial (RCT) is both time-consuming and costly which may take decades. Therefore, it remains largely unknown whether such mediation effects are causal.

Mendelian randomization (MR) is a genetic epidemiological method using genetic variants as instrumental variables for risk factors to explore the unbiased effect on diseases(9). Unlike traditional observational studies, MR studies are less likely to be biased by confounding factors or measurement errors and thus are becoming widely used to investigate the potential causal effect of exposures on outcomes(10). Two-sample MR method means genetic variants for exposure and outcome are extracted from different dataset which makes it more robust in statistical power, but alternative sources of bias may be caused if the two samples used in a study overlap(11). Previous MR studies have proved causal effect of T2DM on stroke, coronary heart disease (CHD) (12, 13), causal effect of T2DM on blood pressure(14), and also causal effects of metabolic factors on CVD(15, 16). Results from these studies indicate that these cardiometabolic factors may partly explain the causal effect of T2DM on CVD, but none of them have quantified the mediation effect. Therefore, to understand how much of the causal effect of T2DM on CVD is mediated by cardiometabolic factors, separately and in combinations, we conducted a two-step, two-sample MR study. We quantified how much of the effects of T2DM on CVD subtypes such as CHD, myocardial infarction (MI) and stroke were mediated through cardiometabolic

factors including SBP, diastolic blood pressure (DBP), low-density lipoprotein (LDL), HDL, TC, TG etc. individually and in all possible combinations by analyzing genome-wide association study (GWAS) summary statistics from international genetic consortia.

2. Methods

2.1 Overall study design

The first step of our two-step MR study is to determine the causal effect of T2DM on each subtype of CVD (CHD, MI and stroke). The second step of our study is to explore and quantify the possible mediation effects of cardiometabolic factors on the causal effect of T2DM on each subtype of CVD.

2.2 Data sources

2.2.1 Genetic instrumental variables for T2DM

We obtained genetic variants for T2DM from a meta-analysis of GWAS which consists of over 16 million genetic variants of European ancestry (17). Sources of participants include DIAGRAM (12,171 cases and 56,862 controls), GERA (6905 cases and 46,983 controls) and UKB datasets (21,147 cases and 434,460 controls) (17).

2.2.2 Genetic instrumental variables for Potential mediators

12 cardiometabolic factors including blood pressure, blood lipids, thyroid function, body mass index (BMI) and insulin sensitivity were selected as potential mediators. Genetic variants of SBP and DBP were both extracted from a genetic analysis of over one million people drawn from UK Biobank (UKB) (18) and the International Consortium of Blood Pressure-Genome Wide Association Studies (ICBP) (19, 20). For TG, hyperthyroidism and hypothyroidism, we obtained data from online public GWAS of European ancestry participants provided by Neale lab and Ben Elsworth through R software TwoSampleMR package (<http://gwas-api.mrcieu.ac.uk/>). We obtained genetic variants of HDL, LDL and TC from a GWAS of 9,961 European participants (21). Genetic variants of VLDL were identified from a GWAS including 19,273 European participants (22). For BMI and WHR, SNPs were extracted from GWAS including ~700,000 and 224,459 European participants, respectively (23, 24). We acquired genetic data sets for insulin sensitivity from a GWAS including 16,753 European participants (25).

2.2.3 Genetic instrumental variables for CVD

GWAS summary statistics for CHD and MI were obtained from a genome-wide association meta-analysis of 48 studies including 60,801 cases and 123,504 controls originating from mixed ancestry (77% from European, 13% and 6% from south and east Asian, others from Hispanic or African American) (26). Genetic variants of stroke were extracted from a multi-ancestry meta-analysis of 29 studies which includes 67,162 cases and 454,450 controls (the number of studies with European ancestry, African ancestry, Asian ancestry and Latin American population GWAS studies was 17, 5, 6 and 1 respectively)

(27). Details of data sources for T2DM, potential mediators and outcomes are shown in Supplementary Table 1. All the genetic variants used as instrumental variables are shown in Supplementary Table 2-14.

2.3 Statistical analysis

2.3.1 Effect of T2DM on CVD subtypes

The causal effects of T2DM on CVD were estimated using a two-sample MR method. We used the inverse-variance weighted (IVW) approach to estimate the causal effect of T2DM on CVD subtypes and each potential mediator. Results were shown using odds ratio (OR) and 95% confidence interval (CI). P-value <0.05 for IVW approach was considered suggestive for potential association.

2.3.2 Effects of T2DM on cardiometabolic factors

Firstly, we used two-sample MR to estimate the effect of T2DM on each cardiometabolic factor. Results were shown using β and 95% CI. P-value <0.05 was considered suggestive for cardiometabolic factors which were causally influenced by T2DM. Only cardiometabolic factors with p-value <0.05 were taken into next procedure and those factors with p-value >0.05 which indicated not statistically significant causal association with T2DM were excluded.

2.3.3 Effects of cardiometabolic factors on CVD subtypes

Secondly, estimates of effects of cardiometabolic factors on CVD subtypes adjusting for T2DM were obtained by regression-based multivariable MR (MVMR) (28). Results were shown using OR and 95% CI. P-value <0.05 was considered suggestive for cardiometabolic factors which had causal effects on CVD. Cardiometabolic factors didn't meet p-value < 0.05 standard were excluded.

2.3.4 Mediation effects of cardiometabolic factors

Further, estimate of effect of T2DM on each cardiometabolic factor was multiplied with estimate of effect of each cardiometabolic factor on CVD subtypes respectively to obtain the mediation effect of each cardiometabolic factor individually. Then we divided the mediation effect by total effect of T2DM on CVD subtypes to obtain the proportion mediated by each mediator. Finally, we took all mediators into account and obtained a causal effect of T2DM on each subtype of CVD after adjusting for all possible mediators. The proportion mediated by all mediators was obtained by subtracting the causal effect of T2DM on each subtype of CVD after adjusting for all mediators from total effect of T2DM on CVD subtypes, then divided the result by the total effect. All the above analyses were performed using R version 4.0.3(The R Foundation for Statistical Computing) through TwoSampleMR package and MendelianRandomization package. Standard errors were calculated using rules derived from Gaussian equation for normally-distributed errors in order to fit different situations such as addition or subtraction and multiplication or division (Supplementary methods).

2.3.5 Sensitivity analyses

We applied other sensitivity analyses including Simple median, Weighted median, MR-Egger regression, MR-PRESSO to detect potential bias from invalid variables and potential pleiotropy, single SNP analysis and leave-one-out analysis to investigate the influence of possible outlying genetic variants. Full details of these methods were shown in Supplementary methods.

3 Results

3.1 Selected SNPs for T2DM

After ruling out SNPs did not meet the standard of genome-wide significance ($p < 5 \times 10^{-8}$) and clumping for those in linkage disequilibrium ($r^2 < 0.001$), 143 SNPs were finally selected for T2DM in our study. Besides, F-statistic of these SNPs were larger than 10 indicating our results were less likely to be biased by weak instruments (29).

3.2 Total effect of T2DM on CVD

We found strong evidence supporting causality of T2DM on subtypes of CVD. Figure 1 shows the total effect of T2DM on CHD, MI and stroke. One-unit higher log odds of T2DM increased 16% risk of CHD (OR: 1.16, 95%CI 1.12-1.21, $p < 0.001$), 15% risk of MI (OR: 1.15, 95%CI 1.10-1.20, $p < 0.001$) and 10% risk of stroke (OR: 1.10, 95%CI 1.06-1.13, $p < 0.001$). Details of genetic associations of T2DM on CVD were shown in Supplementary Table 15-17.

3.3 Effect of T2DM on cardiometabolic factors

Figure 2 shows that one-unit higher log odds of T2DM was associated with increased standard deviation (SD) of SBP ($\beta = 0.77$, 95% CI: 0.49 to 1.04, $p < 0.001$), DBP ($\beta = 0.22$, 95%CI: 0.06 to 0.38, $p = 0.009$), TG ($\beta = 0.08$, 95% CI: 0.02 to 0.14, $p = 0.002$), and WHR ($\beta = 0.05$, 95% CI: 0.03 to 0.07, $p < 0.001$), and was also associated with decreased SD of LDL ($\beta = -0.07$, 95% CI: -0.12 to -0.01, $p = 0.008$), HDL ($\beta = -0.15$, 95% CI: -0.21 to -0.09, $p < 0.001$), TC ($\beta = -0.06$, 95% CI: -0.12 to -0.00, $p = 0.025$) and insulin sensitivity ($\beta = -0.31$, 95% CI: -0.55 to -0.07, $p = 0.012$). We failed to find causal effect of T2DM on BMI ($p = 0.615$), VLDL ($p = 0.168$), hyperthyroidism ($p = 0.213$) and hypothyroidism ($p = 0.159$). Genetic associations of T2DM on each cardiometabolic factor were shown in Supplementary Table 18-29 and those MR results above were shown in details in Supplementary Table 30.

3.4 Effects of cardiometabolic factors on CVD

Figure 3 shows the estimate of causal effect of one SD increase in each cardiometabolic factor on each subtype of CVD after adjusting for T2DM. Estimate of log odds of CHD for one SD increase in SBP, DBP, TG, HDL, WHR and insulin sensitivity was 1.03 (95%CI: 1.02-1.04, $p < 0.001$), 1.05 (95%CI: 1.04-1.06, $p < 0.001$), 1.22 (95%CI: 1.13-1.32, $p < 0.001$), 0.89 (95%CI: 0.65-1.21, $p = 0.521$), 1.06 (95%CI: 0.87-1.30, $p = 0.568$), and 1.00 (95%CI: 0.98-1.01, $p = 0.817$), respectively. The estimated OR of MI for genetically determined one SD increase in SBP, DBP, TG, HDL, WHR and insulin sensitivity was 1.03 (95%CI: 1.02-

1.03, $p < 0.001$), 1.05 (95%CI: 1.04-1.06, $p < 0.001$), 1.22 (95%CI: 1.12-1.33, $p < 0.001$), 0.91 (95%CI: 0.70-1.19, $p = 0.569$), 1.03 (95%CI: 0.83-1.27, $p = 0.812$) and 0.99 (95%CI: 0.97-1.02, $p = 0.503$), respectively. Likewise, one SD increase in genetically determined SBP, DBP, TG, WHR and insulin sensitivity was associated with 3% (OR: 1.03, 95% CI: 1.03-1.04, $p < 0.001$), 4% (OR: 1.04, 95% CI: 1.04-1.05, $p < 0.001$), 0.4% (OR: 1.00, 95% CI: 0.95-1.06, $p = 0.869$), 22% (OR: 0.22, 95% CI: 1.19-1.24, $p = 0.038$), 4% (OR: 1.04, 95%CI: 0.88-1.22, $p = 0.675$) higher risk of stroke and 1% (OR: 0.99, 95%CI: 0.98-1.00, $p = 0.102$) lower risk of stroke, respectively. Regression-based MVMR failed to be performed to estimate the effect of TC and LDL on subtypes of CVD, HDL on stroke for the sake of inadequate SNPs after adjusting for T2DM. Genetic associations of each cardiometabolic factor on each subtype of CVD after adjusting for T2DM were shown in Supplementary Table 31-47.

3.5 Mediation effects of cardiometabolic factors on CVD

After excluding cardiometabolic factors that were not causally influenced by T2DM and those did not have causal effect on CVD subtypes, we took SBP, DBP and TG for mediation analysis. Figure 4 shows the proportion of the effect of T2DM on subtypes of CVD mediated by each cardiometabolic factor included in mediation analysis. For causal effect of T2DM on CHD, the percentage mediated by SBP, DBP and TG was 16% (8%-24%), 7% (1%-13%), and 10% (2%-18%), respectively. The mediation effect of SBP, DBP and TG on MI was estimated to account for 14% (7%-22%), 7% (1%-13%), and 11% (2%-20%), respectively. The proportion of the effect of T2DM on stroke mediated by SBP, DBP and TG was 26% (13%-39%), 10% (2%-19%) and 0.4% (-4%-5%), respectively. Thus, we identified SBP, DBP and TG were main mediators on CHD and MI, SBP and DBP also had significant mediation effect on stroke. Total mediation effect of combination of SBP, DBP and TG on CHD, MI and stroke was 29%, 26% and 13% respectively. Details were shown in Supplementary Table 48-50.

3.6 Sensitivity analyses

Part of results of sensitivity analyses for T2DM on CVD subtypes and three mediators (SBP, DBP and TG) were shown in Table 1. Egger regression results of T2DM-CHD, T2DM-MI, T2DM-DBP and T2DM-TG indicated there might be horizontal pleiotropy existing ($p > 0.05$ with non-zero Egger intercept), however, results from MR-PRESSO outlier-corrected were more consistent with IVW main analysis which are shown in Supplementary Table 51-56. Results of Simple median and Weighted median were mostly statistically significant ($p < 0.05$) which indicated that our results were less likely influenced by weak instrument bias. Other sensitivity analyses including single SNP analysis, leave-one-out analysis both provided consistent results with our main analysis and were shown in Supplementary Table 57-62.

Discussion

In this large-scale multivariable MR study, we estimated that each additional unit of log odds of T2DM was associated with 16% higher risk for CHD, 15% higher risk for MI, and 10% higher risk for stroke. More importantly, approximately one third of excess risk for CVD among T2DM patients was mediated by SBP, DBP, and TG. The most important mediator was elevated SBP, accounting for 16%, 14% and 26% of the

excess risk for CHD, MI, and stroke. Thus, interventions that mitigate these factors might address substantial proportion of excess risk of CVD among T2DM patients.

Previous observational studies have convinced that T2DM was associated with excess CVD risk. A meta-analysis including 698,782 participants from 102 studies concluded that hazard ratios with diabetes were 2.00 (95%CI: 1.83-2.19) for CHD, 2.27 (95% CI: 1.19-2.05) for ischemic stroke (3). Although results were consistent after adjusting for many factors such as sex, smoking status, BMI etc., observational studies might still be biased by other confounders and measurement error. Thus, diabetes participants included in the meta-analysis above might combine other confounders which led to higher CVD risk. Compared with observational studies, results from our study were more consistent with previous MR studies which showed that per unit increase in log-odds of T2DM was associated with increased risk of CHD (OR: 1.11, 95% CI: 1.05-1.17) (30), large-artery stroke (OR: 1.28, 95% CI: 1.16-1.40) (31) and coronary artery disease (OR: 1.63, 95% CI: 1.23-2.07) (13). But none of these studies analyzed causal effect of T2DM on all three main subtypes of CVD and neither had explored the underlying mechanism.

Our study showed that SBP, DBP and TG were main mediators for causal effect of T2DM on CVD. Many previous observational studies have proved that T2DM was associated with blood pressure (32–34) and TG. Besides, results from another MR study also indicated that T2DM had a causal effect on blood pressure (35). However, few studies have investigated whether these metabolic factors had mediation roles in excess CVD risk of T2DM patients.

Previous meta-analyses have documented that metabolic syndrome was an important risk factor for CVD (36, 37). An observational study including 1,038,704 participants in China found that among 85,684 participants with one metabolic disorder at baseline, 28.1% developed additional metabolic disorders which was responsible for higher CVD risk, while among participants without metabolic disorder at baseline only 7.9% had new-onset metabolic disorder (38). Our results further showed significant causal effect of T2DM on cardiometabolic factors and mediation effects of these metabolic factors on the causal effect T2DM on CVD, thus may partly elucidate the unexplained mechanism.

We found that the combined mediation effect of three main mediators including SBP, DBP and TG accounted for 29%, 26%, 13% of total effect on CHD, MI and stroke, implying that intervention on these factors might bring benefit to risk reduction of CVD among T2DM patients. Although blood pressure has already been recommended as treatment target to reduce CVD risk among T2DM patients, opinions on TG were more controversial. Limited by study design or sample size or other factors, clinical trials including the Bezafibrate Infarction Prevention (BIP)(39), the Fenofibrate Intervention and Event Lowering in Diabetes (FIELD)(40) and the Action to Control Cardiovascular Risk in Diabetes Lipid Trial (ACCORD-LIPID)(41) failed to prove fibrate, the TG-reducing drug, could reduce CVD risk among T2DM patients. Results from our study were in favor of new large-scale RCTs investigating whether TG could be new treatment target of T2DM patients to reduce CVD risk and an on-going prospective trial using Pemafibrate is inspiring.

We failed to find evidence for causal associations of T2DM with VLDL and LDL-C, TC, HDL and other lipoprotein fractions. We found no MR studies have been conducted to explore these causal associations in European population, while a small sized one-sample MR study among 9798 participants in East China showed that T2DM increased TC and LDL-C levels (42). The discrepancy may be due to ethnic difference, MR methods and sample size. Importantly, 2016 ESC guidelines mentioned that diabetic dyslipidaemia was combined with multiple plasma lipid and lipoprotein abnormalities and these components were closely linked to each other rather than isolated (43), thus adding difficulties to estimate causal effect of T2DM on a certain fraction of lipoprotein.

Strength And Limitations

To the best of our knowledge, the present study is the first two-sample MR study to identify the mediation effects of cardiometabolic factors of causal effect of T2DM on CVD. Many previous clinical trials have tried to prove the mediation role of these factors (44–46), but limited by short-term follow-up, sample size and most importantly, the influence of confounding factors, thus interpretation of these studies' results became complex. We used two-sample MR to estimate the causal effect of T2DM on subtypes of CVD. The fact that genetic variants were determined at conception before the onset of diseases allows MR to avoid bias from reverse causation and reduce bias from confounding factors which cannot be ignored in observational studies. Besides, in MR studies, participants were life-long exposed to alleles, which is far longer than follow up of RCTs. These unique strengths make MR an effective approach to explore certain causal effect. Compared with one-sample MR, the main advantage of two-sample MR is the increased statistical power because of summary data extracting from different GWAS (47) and less bias caused by pleiotropy because of sensitivity analyses (28). In addition, instrumental variables of T2DM used in our study were obtained from a recently published GWAS. The number of SNPs included in our study was almost three times larger than previous MR studies and explained approximately 10% of the heritability of T2DM (17), while previous studies only accounted for less than 5% (13, 30, 31). All SNPs included have large F-statistics which indicated that our finding was less likely influenced by weak instrument bias. We also applied different MR sensitivity analyses to minimize bias from horizontal pleiotropy or other sources and consistency across these approaches were well evaluated. Finally, we included as many cardiometabolic factors as possible, thus making our results more comprehensive, although we failed to find mediation roles for some cardiometabolic factors.

Horizontal pleiotropy has always been an important source of bias for MR studies. We applied sensitivity analyses which are more robust to horizontal pleiotropy and found consistent results with main analysis from most sensitivity analyses, while MR-Egger results for causal effect of T2DM on CHD and MI indicated potential pleiotropy ($p > 0.05$). However, such pleiotropy for univariable MR also highlights the significance of MVMR to explore roles of mediators. Another limitation of our study is that participants included are mainly European and thus generalize our results to Asian or African population should be more cautious. Besides, we used a linear-regression based MVMR method to estimate the individual mediation effect of each cardiometabolic factor. However, there may be interactions between these mediators which make it possible that one mediator may also affect other mediators thus resulting in

less precise individual effect. Results of our study show that total mediation effect accounted for approximately one third of the total causal effect of T2DM on subtypes of CVD which suggests that there may be some unknown mediators.

Conclusions

By using a two-step, two-sample MR method, our study provides strong evidence for the causal effect of T2DM on development of CVD, and further suggests that approximately one third of excess risk for CVD among T2DM patients is mediated through SBP, DBP and TG, underscoring the importance of large-scale intervention targeting on SBP, DBP and TG which could reduce substantial proportion of CVD risk among T2DM patients.

Declarations

Availability of data and materials

We extracted genetic variants from GWAS datasets including DIAGRAM, GERA, UKB, ICBP and other datasets which could be obtained through R software TwoSampleMR package (<http://gwas-api.mrcieu.ac.uk/>)

Ethics approval and consent to participate

Data included in our study was extracted from previous GWAS datasets and all participants included had written informed consent.

Consent for publication

Not applicable

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Competing interest

There is no competing interest in our study.

Contributors

KC and YT designed this study and also took responsibility for the integrity and accuracy of data and analysis in this study. KC wrote the manuscript and performed data analysis in this study, ZZ, CS, JZ, QZ, TH, ED, YT reviewed and revised the manuscript. All authors had access to data in this study and

contributed to statistical analysis and reviewing the manuscript. KC and YT are the guarantors. All listed authors meet authorship criteria.

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Tables

Table 1. MR sensitivity analyses

Analysis	Nsnp	Effect	Se	P-value	OR
T2DM-CHD					
Simple median	118	0.15	0.02	<0.001	1.16
Weighted median	118	0.11	0.03	<0.001	1.11
MR Egger	118	0.06	0.05	0.267	1.06
MR Egger intercept		0.01	0.00	0.039	
Inverse variance weighted	118	0.15	0.02	<0.001	1.16
T2DM-MI					
Simple median	118	0.15	0.03	<0.001	1.16
Weighted median	118	0.09	0.03	0.001	1.09
MR Egger	118	0.03	0.05	0.499	1.03
MR Egger intercept		0.01	0.00	0.022	
Inverse variance weighted	118	0.14	0.02	<0.001	1.15
T2DM-stroke					
Simple median	118	0.09	0.02	<0.001	1.09
Weighted median	118	0.09	0.02	<0.001	1.09
MR Egger	118	0.08	0.04	0.025	1.09
MR Egger intercept		0.00	0.00	0.817	
Inverse variance weighted	118	0.09	0.02	<0.001	1.10
T2DM-SBP					
Simple median	116	0.65	0.10	<0.001	1.92
Weighted median	116	0.60	0.09	<0.001	1.82
MR Egger	116	0.70	0.32	0.031	2.00
MR Egger intercept		0.01	0.02	0.827	
Inverse variance weighted	116	0.76	0.14	<0.001	2.13
T2DM-DBP					
Simple median	116	0.09	0.06	0.122	1.09
Weighted median	116	0.04	0.05	0.511	1.04
MR Egger	116	0.07	0.19	0.726	1.07

MR Egger intercept		0.01	0.01	0.402	
Inverse variance weighted	116	0.21	0.08	0.012	1.23
T2DM-TG					
Simple median	118	0.07	0.01	<0.001	1.07
Weighted median	118	0.05	0.01	<0.001	1.05
MR Egger	118	0.01	0.06	0.871	1.01
MR Egger intercept		0.01	0.00	0.180	
Inverse variance weighted	118	0.08	0.02	0.002	1.08

MR: Mendelian randomization; Nsnp: Number of SNP; Se: Standard deviation; OR: odds ratio; T2DM: Type-2 diabetes; CHD: Coronary heart disease; MI: Myocardial infarction; SBP: Systolic blood pressure; DBP: Diastolic blood pressure; TG: Triglycerides;

Figures

Figure 1

Estimates of causal effect of T2DM on each subtype of CVD. IVW was used as main analysis for estimating causal effect of T2DM on each subtype of CVD. CHD: Coronary heart disease; MI: Myocardial infarction; T2DM: Type-2 diabetes; IVW: Inverse variance weighted.

Figure 2

Estimate of causal effect of T2DM on cardiometabolic factors. IVW was used as main analysis. One unit increase in logOR of T2DM had positive causality on SBP, DBP, TG and WHR, negative causality on HDL, LDL, TC and insulin sensitivity. Effect of T2DM on BMI, VLDL, hyperthyroidism and hypothyroidism was not statistically significant ($p>0.05$). T2DM: Type-2 diabetes; IVW: Inverse variance weighted; SBP: Systolic blood pressure; DBP: Diastolic blood pressure; TG: Triglycerides; WHR: Waist-hip-ratio; HDL: High-density lipoprotein; LDL: Low-density lipoprotein; TC: Total cholesterol; BMI: Body mass index; VLDL: Very low-density lipoprotein.

Figure 3

Estimate of effect of cardiometabolic factors on each subtype of CVD. MVMR was used to estimate the effect of 1-SD increase in each cardiometabolic factor on each subtype of CVD. (a). Estimate of causal effect of cardiometabolic factors on CHD; (b). Estimate of causal effect of cardiometabolic factors on MI; (c). Estimate of causal effect of cardiometabolic factors on stroke. SD: Standard deviation; CVD: Cardiovascular disease; MVMR: Multivariable Mendelian Randomization; CHD: Coronary heart disease; MI: Myocardial infarction; SBP: Systolic blood pressure; DBP: Diastolic blood pressure; TG: Triglycerides; HDL: High-density lipoprotein; WHR: Waist-hip-ratio.

Figure 4

Proportion of the effect of T2DM on each subtype of CVD mediated by cardiometabolic factors. Proportion of each cardiometabolic factor was calculated by multiplying the effect of T2DM-mediator and the effect of mediator-CVD then divided the result by the effect of T2DM-CVD. T2DM: Type-2 diabetes; CVD: Cardiovascular disease; CHD: Coronary heart disease; MI: Myocardial infarction; SBP: Systolic blood pressure; DBP: Diastolic blood pressure; TG: Triglycerides.

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