



# Genetically Driven Hyperglycemia Increases Risk of Coronary Artery Disease Separately From Type 2 Diabetes

Jordi Merino,<sup>1,2,3</sup> Aaron Leong,<sup>2,4</sup>  
Daniel C. Posner,<sup>5</sup> Bianca Porneala,<sup>4</sup>  
Lluís Masana,<sup>3</sup> José Dupuis,<sup>5,6</sup> and  
Jose C. Florez<sup>1,2,7</sup>

Diabetes Care 2017;40:687–693 | DOI: 10.2337/dc16-2625

## OBJECTIVE

This study tested the hypothesis that genetically raised hyperglycemia increases coronary artery disease (CAD) risk separately from the risk conferred by type 2 diabetes as a whole.

## RESEARCH DESIGN AND METHODS

We conducted a Mendelian randomization (MR) analysis using summary-level statistics from the largest published meta-analyses of genome-wide association studies (GWAS) for fasting glucose (FG) ( $n = 133,010$  participants free of diabetes) and CAD ( $n = 63,746$  case subjects and  $130,681$  control subjects) of predominantly European ancestry. FG-increasing variants associated with type 2 diabetes from the largest GWAS for type 2 diabetes were excluded. Variants with pleiotropic effects on other CAD risk factors (blood lipids, blood pressure, and obesity) were excluded using summary-level data from the largest published GWAS. Data from the Framingham Heart Study were used to validate the MR instrument and to build an FG genetic risk score (GRS).

## RESULTS

In an instrumental variable analysis comprising 12 FG-raising variants, a 1 mmol/L increase in FG revealed an effect-size estimate of 1.43 CAD odds (95% CI 1.14–1.79). The association was preserved after excluding variants for heterogeneity and pleiotropic effects on other CAD risk factors (odds ratio [OR] 1.33 [95% CI 1.02–1.73]). The 12 FG-increasing variants did not significantly increase type 2 diabetes risk (OR 1.05 [95% CI 0.91–1.23]), and its prevalence was constant across FG GRS quintiles ( $P = 0.72$ ).

## CONCLUSIONS

Our data support that genetic predisposition to hyperglycemia raises the odds of CAD separately from type 2 diabetes and other CAD risk factors. These findings suggest that modulating glycemia may provide cardiovascular benefit.

Diabetes, a disease characterized by persistent hyperglycemia, is a global public health crisis affecting 415 million people worldwide (1). Prospective epidemiological data have suggested that individuals with type 2 diabetes have a higher incidence of coronary artery disease (CAD) than those without type 2 diabetes, leading to the consideration of type 2 diabetes as a heart disease risk equivalent for the prediction of future CAD (2). However, type 2 diabetes is a complex disease, typically

<sup>1</sup>Diabetes Unit and Center for Genomic Medicine, Massachusetts General Hospital, Boston, MA

<sup>2</sup>Programs in Metabolism and Medical & Population Genetics, Broad Institute, Cambridge, MA

<sup>3</sup>Vascular Medicine and Metabolism Unit, Research Unit on Lipids and Atherosclerosis, Sant Joan University Hospital, Institut Investigació Sanitària Pere Virgili, Rovira i Virgili University, CIBERDEM, Reus, Spain

<sup>4</sup>Division of General Internal Medicine, Massachusetts General Hospital, Boston, MA

<sup>5</sup>Department of Biostatistics, Boston University School of Public Health, Boston, MA

<sup>6</sup>National Heart, Lung, and Blood Institute's Framingham Heart Study, Framingham, MA

<sup>7</sup>Department of Medicine, Harvard Medical School, Boston, MA

Corresponding author: Jose C. Florez, jcflorez@partners.org.

Received 8 December 2016 and accepted 13 February 2017.

This article contains Supplementary Data online at <http://care.diabetesjournals.org/lookup/suppl/doi:10.2337/dc16-2625/-/DC1>.

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accompanied by increased adiposity, inflammation, oxidative stress, dyslipidemia, and high blood pressure that accelerates atherosclerosis and affects CAD (3). Thus, it is possible that the effect of type 2 diabetes on CAD is not mediated by glycemia per se but by other occult pathogenic risk factors.

Epidemiologic studies have demonstrated that the relation between glycemia and CAD risk starts within the normal blood glucose range (4,5). Yet, randomized clinical trials (RCTs) that successfully lowered glucose levels and delayed the onset of type 2 diabetes in participants with prediabetes have had mixed effects on cardiovascular protection (6–10). Similarly, five separate RCTs including individuals with type 2 diabetes have failed to demonstrate the effectiveness of an intensive glucose-lowering therapeutic approach in reducing CAD events (9–14). Opposite conclusions were drawn from some recent and long-term follow-up studies suggesting that glucose lowering may provide additional cardiovascular benefit (15–19). Inconsistent findings may be partly a result of short intervention and follow-up periods for these studies, the lack of power for trials that were designed for diabetes prevention and not specifically for CAD prevention, or the inclusion of individuals with different duration of type 2 diabetes and diverse clinical characteristics.

A genetic approach can be useful in this scenario. Mendelian randomization (MR) studies may help establish a causal relationship between an exposure (i.e., glycemia) and an outcome (i.e., CAD) because they eliminate reverse confounding and short time exposures by the incorporation of a lifelong exposure (genetic variation randomly assigned at meiosis) that precedes the phenotype and is not affected by the clinical outcome (20). Two recent MR studies have estimated the effect of glycemic traits and type 2 diabetes on CAD risk (21,22). Both studies, however, included a disparate number of fasting glucose (FG)-raising variants associated with type 2 diabetes, thereby allowing for the possibility that such variants have effects on type 2 diabetes globally and not conclusively proving that modulating glycemia per se affects CAD risk.

The observation gleaned from genome-wide association studies (GWAS) that an incomplete overlap exists between genetic

variants that raise glycemia and those that increase type 2 diabetes risk provides a unique opportunity to unravel the relationship between hyperglycemia and CAD separately from type 2 diabetes (23,24). Here, we conducted a discovery and validation genetic instrumental analysis to test the hypothesis that genetically driven hyperglycemia, alone among all other potential metabolic contributors to type 2 diabetes and its metabolic consequences, has an effect on CAD risk.

## RESEARCH DESIGN AND METHODS

### Data Sources and Candidate

#### Instrument Selection

We conducted a MR analysis using summary-level statistics from the largest published meta-analyses of GWAS. Detailed explanations of consortium-specific genotyping, quality control criteria, and the requirements for each phase of the heuristic are included in the Supplementary Data (Supplementary Material 1). Briefly, we identified FG-raising genetic variants at genome-wide significance ( $P < 5 \times 10^{-8}$ ) in the largest published meta-analysis of GWAS of participants without diabetes by the Meta-Analyses of Glucose and Insulin-related traits Consortium (MAGIC) ( $n = 133,010$ ) (24). We excluded FG-associated genetic variants nominally associated with type 2 diabetes ( $P < 0.05$ ) in the largest DIAbetes Genetics Replication And Meta-analyses (DIAGRAMv3) Consortium study ( $n = 34,840$  case subjects and 114,981 control subjects) (25). We then pruned the list of FG-increasing variants by linkage disequilibrium ( $r^2 > 0.2$ ). For each FG-raising variant, we sought summary-level results for CAD from the Coronary ARtery Disease Genome wide Replication and Meta-analysis (CARDIoGRAMplusC4D) ( $n = 63,746$  case subjects and 130,681 control subjects) (26) or CARDIoGRAMv3 ( $n = 22,233$  case subjects and 64,762 control subjects) (27). We limited our analysis to the genetic variants with a significant false discovery rate effect ( $P < 0.05$ ) for each trait. All data sets included participants who were predominantly of European descent.

Next, we interrogated each FG-raising variant for potential heterogeneity and pleiotropic genetic effects on other CAD risk factors. To detect potential pleiotropic associations between included FG-increasing risk variants with factors that may increase or protect against type 2

diabetes, we used the cross-phenotype meta-analysis method (28). This method relies on the hypothesis that each independent genetic variant has multiple phenotypic associations. We prioritized this method over other methods because it is conservative, in that it excludes any variant for which there is statistical evidence of potential pleiotropic effects, irrespective of the strength or the direction of such effects. This is especially relevant for studies conditioning on a common effect (i.e., type 2 diabetes) that will lead to the exclusion of variants associated with factors that may protect against type 2 diabetes (29). Consequently, an MR analysis that uses this procedure and yields statistically significant results is likely to reflect a true causal association between exposure and outcome.

A detailed explanation of the pleiotropy analysis source criteria and cohort-specific GWAS appears in the Supplementary Data (Supplementary Material 2). Briefly, we included cohort-specific summary level results from the largest GWAS meta-analyses for each trait, where such data were publicly available: 1) blood lipids (including LDL cholesterol, HDL cholesterol, and triglycerides) (30), 2) systolic and diastolic blood pressure (31), and 3) BMI (32). For our polygenic MR instrument, we considered the most restrictive pleiotropic threshold of no pleiotropic associations ( $P < 0.05$ ) on other CAD risk factors. Details of pleiotropy for each of the interrogated FG-increasing genetic variants across CAD risk factors are described in Supplementary Table 1. Finally, as an additional check for potential pleiotropy, we examined the association of our aggregate nonpleiotropic MR instrument with each of these metabolic traits.

To estimate effect sizes for FG-increasing risk variants on type 2 diabetes risk, we conducted a genetically derived approximation including 1) a subset of FG-raising variants not associated with type 2 diabetes ( $n = 12$ ), 2) a set of previously reported FG-associated variants (ignoring possible association with type 2 diabetes,  $n = 32$ ), and 3) a subset of previously reported variants excluding our subset of nonoverlapping variants ( $n = 20$ ). Details of the included variants in each MR analysis are reported in Supplementary Table 2.

### Power Calculations

Power calculations were performed using two-stage least squares regression

estimates for MR studies (33). For the causal estimate of increased FG on CAD, with the available sample size of 63,746 CAD case subjects, we had 80% statistical power to detect differences in CAD odds higher than 1.03 on the causal effect between raised FG and CAD (type I error rate set at 0.05). The sample size of DIAGRAMv3 ( $n = 34,840$  case subjects) gave us 80% statistical power to detect an effect of equivalent magnitude of type 2 diabetes odds higher than 1.04 on the causal effect between raised FG and type 2 diabetes (type I error rate set at 0.05).

### Instrument Validation

MR validation was conducted using individual-level data from Offspring and Third Generation cohorts from the Framingham Heart Study (FHS) in 5,113 participants free of diabetes (34,35). We estimated the contribution of risk score variants to FG variance and FG heritability. We used genotyped variants with a genotyping success rate of  $\geq 0.95$  and variants in Hardy-Weinberg equilibrium ( $P > 1 \times 10^{-4}$ ). When not directly genotyped, variants were imputed using HapMap2 or 1000 Genomes Project reference panels by MACH software (<http://csg.sph.umich.edu/abecasis/MaCH/download/>) using previously described filters (23). Uncertainty in imputation was accounted for by using the dosage genotype (i.e., expected number of coded allele) in a linear mixed-effect model implemented in the lmeKin function of the R kinship2 package (available at <http://cran.r-project.org/web/packages/kinship2>). To ensure high-quality imputed genotypes, we used an  $r^2$  value threshold of 0.95, representing an approximate correlation with the true genotype of 0.97. Details on genotypes imputation and quality metrics are described in the Supplementary Data (Supplementary Material 3 and Supplementary Table 3).

### FG Genetic Risk Score

The FG genetic risk score (GRS) was calculated on the basis of the 12 identified FG-increasing genetic variants not associated with type 2 diabetes. On the assumption of an additive genetic effect, the risk score summed risk alleles weighted by their respective effect sizes on FG ( $\beta$ -estimate coefficients) in the MAGIC data set (24). The score was then rescaled to range from 0 to 24 units so that a

participant with two risk alleles of each variant would have a GRS of 24 units. Higher scores indicated a higher genetic predisposition to increased FG.

### Statistical Analyses

To obtain an estimated effect of FG on CAD, the causal effect ( $\alpha$ ) instrumented by each FG-raising genetic variant was calculated as the ratio of  $\beta$ -coefficients for CAD and FG ( $\alpha = \beta_{CAD}/\beta_{FG}$ ), and the SE was calculated as ( $SE = SE_{CAD}/\beta_{FG}$ ). Regression analyses from the Genetics ToolboX package (available at <http://cran.r-project.org/web/packages/gtx>) were used to obtain the overall instrumental effect-sizes of the exposure on the outcome. This approach, equivalent to performing an inverse-variance weighted meta-analysis of ratio estimates across variants, allowed us to minimize the potential for heteroscedasticity influencing our overall causal estimate because the method routinely lowers the weight of lower frequency variants as they have larger SEs (36).

Heterogeneity of the overall MR instrument was assessed using the  $Q$  statistic and reported as a heterogeneity  $P$  value. Cook's distance was used to estimate heterogeneity of the individual FG-increasing risk variants. We also interrogated each FG-raising genetic variant for potential pleiotropic nominal associations with other CAD risk factors ( $P < 0.05$ ). The overall effect-sizes on CAD risk using the polygenic MR instruments were reported as odds ratios (ORs) and 95% CIs of OR per 1 mmol/L increase in FG levels. We took two-sided  $P < 0.05$  to denote evidence against the null hypothesis in MR analyses.

To validate our MR instrument, we estimated the contribution of risk score variants to FG variance and FG heritability in a linear mixed-effect model with an additive genetic model, age and sex covariates, and random effects to account for familial correlation in participants without diabetes from the Offspring and Third Generation cohorts from FHS. To measure the combined effect of the 12 FG-increasing variants on FG, we computed quintiles of the 12-variant GRS. Differences in FG between GRS quintiles were analyzed in linear mixed-effects models accounting for familial correlation among participants. To assess whether type 2 diabetes prevalence differed across GRS

quintiles, we used specified generalized estimating equations models, after including available data from 724 individuals with type 2 diabetes from the FHS. Differences in other clinical characteristics were assessed across quintiles using the combined cohort of participants with and without diabetes from the FHS. Participants with missing phenotype or covariates were excluded from the analysis. In all models, we accepted the GRS was associated with the dependent variable if the omnibus test for GRS quintiles was significant ( $P < 0.05$ ). R 3.2.0 software (<https://www.r-project.org/>) and Stata 12.0 software (StataCorp LP, College Station, TX) were used for the statistical analyses.

### RESULTS

We identified 12 independent FG-raising genetic variants at genome-wide significance that were not associated with type 2 diabetes. Selection criteria requirements for each phase of the heuristic to arrive at those 12 independent FG-increasing genetic variants are shown in Supplementary Fig. 1. Details on the risk alleles, frequency,  $P$  values, sample sizes, and magnitude of the effect on FG, type 2 diabetes, and CAD are presented in Table 1.

In an instrumental variable analysis using a polygenic instrument of 12 FG-raising variants, a 1 mmol/L increase in FG raised CAD odds by 43% (OR 1.43 [95% CI 1.14–1.79]; heterogeneity  $P = 0.02$ ) (Fig. 1). One variant introduced heterogeneity in the MR instrumental analysis (AMT, rs11715915; Cook's distance = 0.66). After excluding this variant, we showed that the association of increased FG and CAD was preserved (OR 1.34 [95% CI 1.07–1.68]; heterogeneity  $P = 0.88$ ) (Supplementary Fig. 2). Next, we interrogated each of the FG-raising variants for potential pleiotropic effects on other CAD risk factors, including blood lipids, blood pressure, and obesity. The use of a conservative pleiotropy threshold ( $P < 0.05$ ) resulted in five FG risk-increasing variants being treated as instrumental variables. A 1 mmol/L increase in FG derived from the MR instrument set of five FG-raising variants revealed an OR of 1.33 (95% CI 1.02–1.73) for CAD (Fig. 2). The MR instrument constructed with these five nonpleiotropic variants was not associated with higher LDL cholesterol, triglycerides, systolic or diastolic

**Table 1—Characteristics of genetic variants considered for use in Mendelian randomization analysis of the effect of FG on CAD**

Chr	Position	SNP	Genes	EA	MAF	OR FG	OR CAD	OR T2D	P value FG (n = 133,010)	P value CAD (n = 194,427)	P value T2D (n = 149,821)
2	169 471 394	rs560887	<i>G6PC2</i>	C	0.33	1.07	1.02	0.97	$1.40 \times 10^{-178}$	$1.51 \times 10^{-1}$	$1.10 \times 10^{-1}$
3	172 195 984	rs1280	<i>SLC2A2</i>	T	0.14	1.03	1.00	1.04	$8.56 \times 10^{-18}$	$6.36 \times 10^{-1}$	$6.60 \times 10^{-2}$
3	49 430 334	rs11715915	<i>AMT</i>	G	0.27	1.01	1.05	1.00	$4.90 \times 10^{-8}$	$6.29 \times 10^{-06}$	$5.30 \times 10^{-1}$
5	95 565 204	rs4869272	<i>PCSK1, MIR583</i>	T	0.32	1.02	0.99	1.01	$1.02 \times 10^{-15}$	$5.81 \times 10^{-1}$	$1.81 \times 10^{-1}$
9	110 719 930	rs16913693	<i>IKBKAP</i>	T	0.02	1.04	1.03	0.96	$3.51 \times 10^{-11}$	$3.50 \times 10^{-1}$	$5.30 \times 10^{-1}$
10	113 032 083	rs10885122	<i>ADRA2A</i>	G	0.11	1.03	1.02	1.02	$6.32 \times 10^{-17}$	$2.14 \times 10^{-1}$	$5.30 \times 10^{-2}$
11	47 303 299	rs11039182	<i>MADD</i>	T	0.31	1.02	1.00	1.01	$4.82 \times 10^{-22}$	$8.94 \times 10^{-1}$	$4.42 \times 10^{-1}$
11	61 360 086	rs174576	<i>FADS1</i>	T	0.37	1.02	1.02	1.03	$1.2 \times 10^{-18}$	$8.93 \times 10^{-2}$	$1.24 \times 10^{-1}$
12	131 551 691	rs10747083	<i>P2RX2</i>	A	0.25	1.01	1.02	1.01	$7.57 \times 10^{-09}$	$3.16 \times 10^{-1}$	$7.57 \times 10^{-1}$
13	27 385 599	rs11619319	<i>PDX1</i>	A	0.21	1.02	1.01	0.99	$1.33 \times 10^{-15}$	$3.37 \times 10^{-1}$	$5.86 \times 10^{-1}$
14	99 909 014	rs3783347	<i>WARS</i>	G	0.22	1.02	1.02	1.00	$1.32 \times 10^{-10}$	$1.66 \times 10^{-1}$	$8.47 \times 10^{-1}$
20	22 505 099	rs6113722	<i>FOXA2</i>	G	0.04	1.04	1.00	1.00	$2.49 \times 10^{-11}$	$8.75 \times 10^{-1}$	$9.42 \times 10^{-1}$

Chr, chromosome; EA, effect allele; MAF, minor allele frequency; SNP, single nucleotide polymorphism; T2D, type 2 diabetes.

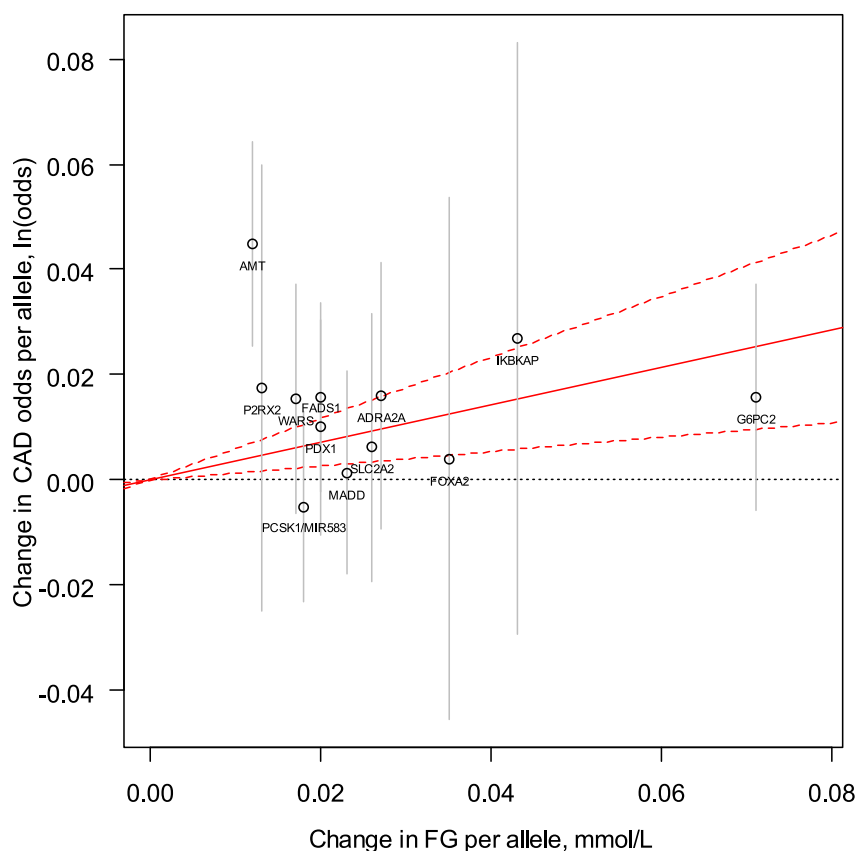
blood pressure, and BMI or with lower HDL cholesterol ( $P > 0.05$ ).

We also showed that the polygenic instrument of 12 FG-raising variants did

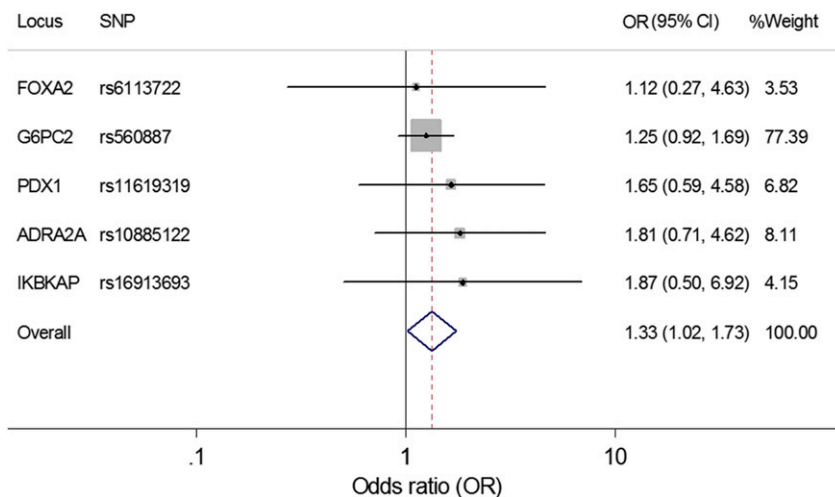
not significantly increase type 2 diabetes odds (OR 1.05 [95% CI 0.91–1.23]; heterogeneity  $P = 0.09$ ) (Supplementary Fig. 3). As expected, in an independent MR

analysis including all 32 FG-raising variants reported to date, a 1 mmol/L increase in FG increased type 2 diabetes odds by approximately twofold (OR 1.94 [95% CI 1.77–2.12]) (Supplementary Fig. 4). The estimated effect size of increasing FG on type 2 diabetes was even stronger after our subset of 12 FG-raising variants was removed (OR 2.74 [95% CI 2.44–3.07]) (Supplementary Fig. 5). The contrasts between these two last estimated effect sizes on type 2 diabetes odds were statistically significant ( $P = 0.03$ ).

In a validation study of individuals without diabetes from the FHS Offspring and Third Generation cohorts, the 12 FG-raising variants MR instrument explained a substantial proportion (5.03%) of the inherited variation in FG. The mean FG concentration was higher for those individuals in the top quintile of the GRS compared with individuals in the lower score ( $\beta = 0.11$ , SE = 0.02;  $P = 6.25 \times 10^{-8}$ ) (Fig. 3). After available data from 724 individuals with type 2 diabetes from the FHS were included, the prevalence of type 2 diabetes did not differ across FG GRS quintiles (Q1 = 13.1% vs. Q5 = 11.9%;  $P = 0.72$ ) (Fig. 3), and the difference in FG across GRS quintiles was attenuated (Supplementary Table 4). A complete description of clinical characteristics according to FG GRS quintiles is detailed in (Supplementary Table 4). We did not observe statistical differences across FG GRS quintiles for other CAD risk factors, including age, sex, metabolic syndrome, BMI, blood lipids, or blood pressure. Similarly, continuous FG GRS did not affect type 2 diabetes risk (OR 0.98 [95% CI 0.94–1.02]) after adjusting



**Figure 1—**Estimate effect size of raised FG on CAD odds. Effect of FG-raising genetic variants (mmol/L) on CAD odds obtained from publicly available data from MAGIC and CARDIoGRAM Consortia. Each white dot represents a genome-wide FG-raising variant not associated with type 2 diabetes. The association of each variant with CAD (logarithmic OR transformation,  $\ln(\text{OR})$ ) is denoted in the y-axis, and the association with FG is denoted by the x-axis. The red lines illustrate regression and 95% CI of FG on CAD. The effect of the polygenic instrument comprising all 12 FG-increasing variants increased the odds of CAD risk per 1 mmol/L increase in FG (OR 1.43 [95% CI 1.14–1.79]).



**Figure 2**—MR estimate of raised FG on odds of CAD is shown in subgroup analyses after pleiotropic exclusion computed using five FG-raising variants. Shown for each FG-raising genetic variant are the estimates of raised FG on CAD odds obtained from publicly available data from MAGIC and CARDIoGRAM Consortia (blue diamond). Also, shown for each FG-increasing genetic variant is the estimate of the effect and the inverse variance weight (percentage proportional to the size of the gray square) and the 95% CI of the estimate. Each FG-raising genetic variant was tested for evidence of pleiotropic associations with other CAD risk factors using the cross-phenotype meta-analysis method at the most conservative threshold that excluded variants with nominal pleiotropic associations ( $P < 0.05$ ). The MR instrument comprising five FG-raising variants increased odds of CAD risk by 33% per 1 mmol/L increase in FG (OR 1.33 [95% CI 1.02–1.73]). SNP, single nucleotide polymorphism.

for age, age squared, and sex (Supplementary Table 5).

**CONCLUSIONS**

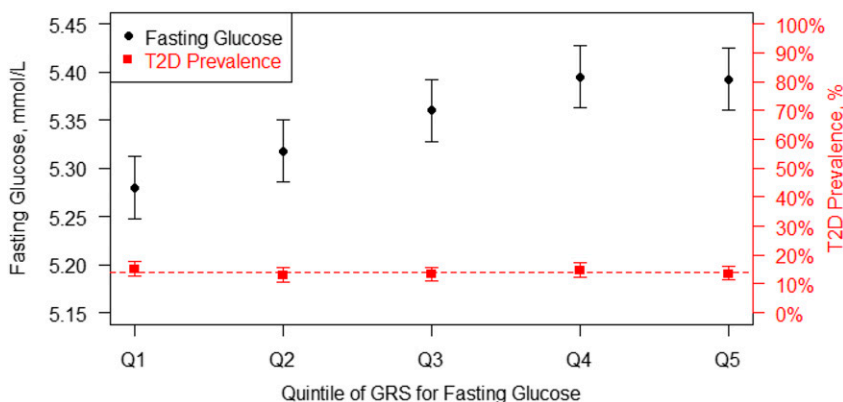
Whether hyperglycemia raises CAD risk separately from concomitant patho-

physiological processes of type 2 diabetes is uncertain. Our MR investigation confirmed that increased FG, determined by FG-increasing genetic variants that were not associated with type 2 diabetes risk, raised CAD risk. We report

an increase in CAD odds of 43% per 1 mmol/L of raised FG. The association was similar when using a genetic instrument composed of FG-associated genetic variants that did not exhibit heterogeneous effects and did not have pleiotropic effects on other CAD risk factors (33% higher CAD odds per 1 mmol/L increase in FG).

FG is one of the diagnostic criteria for type 2 diabetes. However, clinical data suggest that not all modest physiological elevations in FG will be associated with type 2 diabetes (37), and even after 15 years of exposure to increased FG concentrations, not all individuals progress to type 2 diabetes (10). Although type 2 diabetes has been long recognized as a CAD risk factor, the complex interaction of several metabolic alterations supports the difficulty in determining whether the glycemic component has an effect on CAD risk (38). Epidemiologic studies in this area are subject to confounding, and RCTs for glycemic control have been inconclusive. Genetic analyses that incorporate a GRS as a polygenic instrument in an MR framework can capture an adequate proportion of variance in an endophenotype and establish causality for that trait. The key novel point of the current study is the inclusion of nonoverlapping FG and type 2 diabetes variants. In this study, we analyzed one component of type 2 diabetes, hyperglycemia, while attempting to exclude other components associated with a complex disease process that involves mechanisms beyond hyperglycemia. Our study supports the hypothesis that glucose elevation per se is a key contributor to increased CAD.

A clinical implication of our findings is that they lend credence to the notion that lowering glucose may confer cardiovascular benefit even among individuals without diabetes. Prospective epidemiological data have consistently shown that even small changes in blood glucose can have an effect on cardiovascular morbidity and mortality in healthy participants (39). In an extended meta-regression analysis, a linear relationship with no threshold effect was observed between FG and CAD risk (the relative cardiovascular event risk reduction was 1.33 compared with FG concentrations of 4.2 vs. 6.1 mmol/L) (40). In addition, intensive glucose-lowering therapy reduced the risk of myocardial infarction in people with newly diagnosed



**Figure 3**—FG concentrations and type 2 diabetes (T2D) prevalence according to individual FG genetic risk score quintiles. Variation in FG concentrations and type 2 diabetes prevalence depending on the number of risk alleles identified at the FG loci, weighted by effect size in an aggregate genotype score for the FHS Offspring examination 8 and Third Generation examination 2 participants. FG GRS quintiles are represented on the x-axis. The left y-axis displays FG concentrations (mean  $\pm$  1.96  $\times$  SE) in individuals without diabetes according to the GRS quintiles (black dots). The right y-axis shows type 2 diabetes prevalence across GRS quintiles ( $n = 724$  participants with type 2 diabetes). The FG difference ( $\beta$ -coefficient) between the top and bottom quintiles was 0.11 mmol/L (SE 0.02;  $P = 6.25 \times 10^{-8}$ ). Type 2 diabetes prevalence was constant across FG GRS quintiles (Q1 = 13.1% vs. Q5 = 11.9%,  $P = 0.72$ ). In all models,  $P$  values were obtained by the omnibus test for GRS quintiles.

type 2 diabetes (16) and in patients with established type 2 diabetes by 15 to 20% (15). A meta-analysis of large RCTs found intensive glucose-lowering was associated with a significant 9% reduction in CAD events (41).

Although the current study was not designed to elucidate a potential mechanism of action, our findings concur with previous observations that plasma glucose elevations can directly aggravate structural changes in the arterial wall by a variety of mechanisms, including endothelial dysfunction, vascular smooth muscle cell proliferation, and an inflammatory phenotype change in macrophages (42). Consequently, a lifetime exposure to increased FG is likely to have detrimental effects on cardiovascular disease. A recent pharmacogenomic study suggested that the low-frequency missense variant Ala316Thr (rs10305492) in the glucagon-like-peptide 1 receptor gene (*GLP1R*) was associated with lower FG and conferred protection against CAD risk, highlighting the role of hyperglycemia on CAD risk and suggesting a glucose-driven mechanism (43). We note that in our MR instrument, the variant rs560887 at the *G6PC2* locus, the strongest common genetic determinant of FG (44), was the main contributor of the MR effect estimate of FG on CAD. *G6PC2* encodes the glucose-6-phosphatase catalytic 2 subunit, which regulates glycemia by opposing the action of glucokinase (GCK) in pancreatic  $\beta$ -cells, thereby modulating glycolytic flux and glucose-stimulated insulin secretion (44). Individuals with *GCK* mutations (responsible for maturity-onset diabetes of the young, type 2), who have mild hyperglycemia from birth, do not seem to be at increased risk of CAD compared with unaffected family members (45). However, early diagnosis of diabetes in this population may have intensified treatment of other potent CAD risk factors, such as hypercholesterolemia, thereby conferring additional cardiovascular protection in mutation carriers (45).

Our results should be interpreted with caution because other unmeasured factors (e.g., those related to inflammation or oxidative stress) could bias our estimate of elevated FG on CAD. In addition, given different sample sizes of GWAS datasets for the various traits and a uniform *P* value threshold of 0.05

to establish pleiotropy, the effect size detected to make this determination will differ per trait; nevertheless, we elected to choose a metric establishing a uniform likelihood of deviation from chance over a metric intending to capture similar effect sizes across phenotypes. We also recognize that nongenetic factors may have a more dominant role in FG and CAD risk. MR studies make several assumptions, including confounding by pleiotropy and linkage disequilibrium, population structure, or weak genetic instruments (46). Confounding by pleiotropy and linkage disequilibrium was properly addressed, but we cannot affirm that our results apply to other ethnic groups because we conducted our analyses mainly in populations of European descent.

Our validation analysis showed that our genetic instrument explains a small proportion of FG variance, although the magnitude of variance explained is aligned with previous observations (23,24) and is sufficient to capture the effects of genetically explained FG, especially for individuals without diabetes. Nevertheless, we cannot rule out that a genetic instrument constructed with a different set of undiscovered variants, derived from deeper genotyping platforms or other populations, might yield disparate results. Our MR variable analysis also assumed a linear relationship between FG and CAD. We note that FG may have a nonlinear relationship with CAD, but this is typically seen for individuals with low glucose levels, which represent a minority of the general population (2). Finally, we cannot rule out the possibility that FG-raising variants may have an independent effect on FG and CAD or an effect on CAD that varies with the extent of glycemic exposure.

In conclusion, our findings quantify the causal relationship between increased FG and CAD risk beyond the genetic effect of type 2 diabetes and other major CAD risk factors. We demonstrated that a 1 mmol/L increase in FG increased the odds of CAD by 33%. Our results provide additional evidence for the design and interpretation of clinical trials of interventions specifically designed to lower glycemia on CAD outcomes.

**Acknowledgments.** The authors thank publicly available genomic consortia (MAGIC, DIAGRAM,

CARDIoGRAMplusC4D, and CARDIoGRAMv3) for access to their data.

**Funding.** This work was partially supported by the National Heart, Lung, and Blood Institute's FHS (Contract No. N01-HC-25195). J.M. is supported by an individual fellowship funded by the Horizon 2020 Marie Skłodowska-Curie Actions of the European Commission (H2020-MSCA-IF-2015-703787). A.L. is supported by a Canadian Diabetes Association postdoctoral research fellowship. J.D. is partially supported by National Institutes of Health grant R01-DK-078616. J.C.F. is supported by an MGH (Massachusetts General Hospital) Research Scholars Award.

**Duality of Interest.** No potential conflicts of interest relevant to this article were reported.

**Author Contributions.** J.M., A.L., D.C.P., B.P., L.M., J.D., and J.C.F. participated in the interpretation of data, the drafting of the manuscript and its revisions, and final approval of the manuscript. J.M., D.C.P., and B.P. acquired and analyzed the data. J.M., J.D., and J.C.F. participated in the design and conception of the study. J.M., J.D., and J.C.F. are the guarantors of this work and, as such, had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

**Prior Presentation.** Parts of this study were presented in abstract form at the 52nd European Association for the Study of Diabetes Annual Meeting, Munich, Germany, 12–16 September 2016, and at the Endocrine Society's 98th Annual Meeting and Expo, Boston, MA, 1–4 April 2016.

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