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Nonfasting Glucose, Ischemic Heart Disease, and Myocardial Infarction

A Mendelian Randomization Study

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Objectives The purpose of this study was to test whether elevated nonfasting glucose levels associate with and cause ischemic heart disease (IHD) and myocardial infarction (MI).

Background Elevated fasting plasma glucose levels associate with increased risk of IHD, but whether this is also true for nonfasting levels and whether this is a causal relationship is unknown.

Methods Using a Mendelian randomization approach, we studied 80,522 persons from Copenhagen, Denmark. Of those, IHD developed in 14,155, and MI developed in 6,257. Subjects were genotyped for variants in *GCK* (rs4607517), *G6PC2* (rs560887), *ADCY5* (rs11708067), *DGKB* (rs2191349), and *ADRA2A* (rs10885122) associated with elevated fasting glucose levels in genome-wide association studies.

Results Risk of IHD and MI increased stepwise with increasing nonfasting glucose levels. The hazard ratio for IHD in subjects with nonfasting glucose levels ≥11 mmol/l (≥198 mg/dl) versus <5 mmol/l (<90 mg/dl) was 6.9 (95% confidence interval [CI]: 4.2 to 11.2) adjusted for age and sex, and 2.3 (95% CI: 1.3 to 4.2) adjusted multifactorially; corresponding values for MI were 9.2 (95% CI: 4.6 to 18.2) and 4.8 (95% CI: 2.1 to 11.2). Increasing number of glucose-increasing alleles was associated with increasing nonfasting glucose levels and with increased risk of IHD and MI. The estimated causal odds ratio for IHD and MI by instrumental variable analysis for a 1-mmol/l (18-mg/dl) increase in nonfasting glucose levels due to genotypes combined were 1.25 (95% CI: 1.03 to 1.52) and 1.69 (95% CI: 1.28 to 2.23), and the corresponding observed hazard ratio for IHD and MI by Cox regression was 1.18 (95% CI: 1.15 to 1.22) and 1.09 (95% CI: 1.07 to 1.11), respectively.

Conclusions Like common nonfasting glucose elevation, plasma glucose-increasing polymorphisms associate with increased risk of IHD and MI. These data are compatible with a causal association. (J Am Coll Cardiol 2012;59:2356–65)

Elevated fasting plasma glucose levels are associated with increased risk of ischemic heart disease (IHD) and myocardial infarction (MI) in subjects with and without diabetes mellitus (1,2), but it is unclear whether this is also true for nonfasting levels. Although intensive glycemic control may reduce the risk of IHD and MI (3), it is unclear whether this risk reduction is due to reduced glucose levels per se or to an improvement of concomitant obesity, dyslipidemia, and hypertension.

To study a potential causal relationship a Mendelian randomization approach can be used to circumvent confounding and reverse causation (4). This approach uses the fact that genetic glucose-increasing variants are randomly assorted during gamete formation, like patients are randomized to placebo or active treatment in intervention trials; and, if genotypes associated with higher plasma glucose levels also are associated with increased risk of IHD and MI compared to genotypes associated with lower levels, it follows that this likely is a causal association. To use this approach, we genotyped variants in *GCK* (rs4607517) (5), *G6PC2* (rs560887) (5–7), *ADCY5* (rs11708067) (8), *DGKB*

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(rs2191349) (6), and *ADRA2A* (rs10885122) (8), which were selected as the variants associated with the largest effects on fasting glucose levels in genome-wide association studies specifically aiming to identify variants associated with plasma glucose levels, and not previously associated with IHD and MI (9). Furthermore, the genetic variants have no major effects on other risk factors and, therefore, can be used to study the impact of longstanding differences in plasma glucose levels without known pleiotropic effects.

We tested the hypothesis that there is a potential causal association between elevated nonfasting glucose levels and increased risk of IHD and MI. As most subjects are in the nonfasting state the majority of a 24-h cycle, and thus exposed to higher glucose levels than observed through fasting measurements, it may be more important to study nonfasting than fasting glucose levels. First, we tested whether elevated nonfasting glucose levels are associated with increased risk of IHD; second, whether the selected genotypes (5,8,10,11) are associated with elevated nonfasting glucose levels; and finally, whether genotypes are associated with increased risk of IHD and MI both as single site genotypes and combined in instrumental variable analyses.

Methods

Subjects. Studies were approved by institutional review boards and Danish ethical committees, and conducted according to the Declaration of Helsinki. Participants were all white persons and of Danish descent, and gave informed consent. None appeared in >1 study.

The CCHS. The CCHS (Copenhagen City Heart Study) is a prospective study of the general population initiated in 1976 to 1978 with follow-up examinations in 1981 to 1983, 1991 to 1994, and 2001 to 2003, and endpoints ascertained from 1976 through May 2011 (12). Participants were selected to reflect the adult Danish population ages 20 to 80+ years. Data were obtained from a questionnaire, a physical examination, and from blood samples at each examination. Baseline nonfasting plasma glucose levels were available on 16,568 participants attending the 1976 to 1978, 1981 to 1983, 1991 to 1994, and/or 2001 to 2003 examinations. Baseline was defined as the first examination a subject participated in. Blood samples for deoxyribonucleic acid (DNA) extraction were available on 10,603 participants attending the 1991 to 1994 and 2001 to 2003 examinations.

The CGPS. The CGPS (Copenhagen General Population Study) is a cross-sectional/prospective study of the general population initiated in 2003 with ongoing enrollment (13–15) and endpoints ascertained from 1976 through May 2011. Participants were selected and examined exactly as in the CCHS. At the time of genotyping, 48,614 subjects had been included.

The CIHDS. The CIHDS (Copenhagen Ischemic Heart Disease Study) comprises 5,109 patients from the greater Copenhagen area referred for coronary angiography in 1991 to 2010 and 10,231 controls without IHD ascertained as for the CGPS (13–15). In addition to a diagnosis of IHD, these patients also had stenosis/atherosclerosis on coronary angiography and/or a positive exercise electrocardiography test, and/or MI.

Abbreviations and Acronyms

BMI = body mass index
CI = confidence interval
HDL = high-density lipoprotein
IHD = ischemic heart disease
MI = myocardial infarction

Ischemic heart disease and myocardial infarction. In all studies, information on diagnosis of IHD and MI according to WHO International Classification of Diseases (ICD) (ICD8: 410–414 and 410; and ICD10: I20–I25 and I21) was collected and verified from 1976 through May 2011 by reviewing all hospital admissions and diagnoses entered in the national Danish Patient Registry and all causes of death in the national Danish Causes of Death Registry. Ischemic heart disease was angina pectoris and MI, the latter determined on the basis of characteristic chest pain, electrocardiographic changes, and/or elevated cardiac enzymes following the changes in diagnostic criteria over time (16). Follow-up was 100% complete, that is, no subject was lost to follow-up in any study.

Genotypes. Genotyping for *GCK* (rs4607517), *G6PC2* (rs560887), *ADCY5* (rs11708067), *DGKB* (rs2191349), and *ADRA2A* (rs10885122) was by TaqMan, ABI Prism 7900HT Sequence Detection System (Applied Biosystems, Foster City, California). Each run included a known noncarrier, a heterozygous, and a homozygous control verified by sequencing. After 2 reruns, call rates for genotypes where >99.9% for all assays. We also genotyped the *MTNR1B* (rs10830963) variant, but it did not associate with nonfasting glucose levels (observed per allele effect, nonfasting: −0.025 [SEM 0.01]) as reported for fasting glucose levels in genome-wide association studies (per allele effect in literature: 0.072 [SEM 0.005]) (5,8), and were therefore excluded from further analysis.

Biochemical analyses. Colorimetric assays were used to measure nonfasting plasma glucose, total cholesterol, and high-density lipoprotein (HDL) cholesterol (Konelab, Boehringer Mannheim, Germany). Blood samples were taken at random irrespective of content of or of time since the last meal.

Other covariates. Body mass index (BMI) was weight (kg) divided by height squared (m^2); metabolic syndrome was defined as fulfilling 3 of the following 5 criteria: waist circumference ≥102 cm for men and ≥88 cm for women, triglycerides ≥1.7 mmol/l (150 mg/dl) or drug treatment for elevated triglycerides, HDL <1.0 mmol/l (40 mg/dl) in men and <1.3 mmol/l (50 mg/dl) in women or drug treatment for low HDL, systolic blood pressure ≥130 mm Hg and/or diastolic blood pressure ≥85 mm Hg or antihypertensive drug treatment, fasting glucose ≥5.56 mmol/l (100 mg/dl), or antidiabetic medication (17); diabetes mellitus was self-reported diabetes, use of antidiabetic medication, a nonfasting plasma glucose >11.0 mmol/l, and/or hospitalization or death due to diabetes (ICD8: 249–250;

ICD10: E10–E11, E13–E14); hypertension was defined as systolic blood pressure ≥140 mm Hg (≥135 mm Hg for diabetic subjects), diastolic blood pressure ≥90 mm Hg (≥85 mm Hg for diabetics), and/or use of antihypertensive medication prescribed specifically for hypertension; current smoking, menopause status, and statin use were also self-reported.

Statistical analyses. Data were analyzed by 2 authors (M.B. and B.G.N.) using Stata/IC version 11.1 (StataCorp, College Station, Texas). Two-sided $p < 0.05$ was significant. For trend tests using Cuzick nonparametric test and extension of a Wilcoxon rank sum test, groups of subjects classified by nonfasting glucose levels, genotypes, or number of alleles were ranked according to increasing nonfasting glucose levels and coded as 0, 1, 2, 3, and so forth.

First, to test whether nonfasting glucose associate with increased risk of IHD and MI, Kaplan-Meier curves were used to estimate cumulative incidence, and Cox regression models with age as time scale and left truncation (delayed entry) were used to estimate hazard ratios for IHD and MI in the prospective CCHS. Analyses were conducted using results from each participant's first glucose measurement in the 1976 to 1978, 1981 to 1983, 1991 to 1994, or 2001 to 2003 examinations (i.e., baseline), and data from the serial examinations of other risk factors were used as time-dependent covariates for multifactorial adjustment. Risk of IHD and MI was estimated as a function of nonfasting glucose levels in groups of 1-mmol/l (18-mg/dl) increases versus <5.0 mmol/l (<90 mg/dl) and as a continuous variable, and was corrected for regression dilution bias using a factor of 0.45 (Online Figs. 1 and 2) (18). Analyses were adjusted for: 1) age and sex; 2) multifactorially for age, sex, current smoking, menopause, and statin use; 3) multifactorially including BMI; 4) multifactorially including total cholesterol and HDL cholesterol; 5) multifactorially including hypertension; and 6) multifactorially including all of the above.

Second, to test whether genotype associate with elevated nonfasting glucose levels, per allele effects of genotypes were calculated in the CCHS and CGPS combined to obtain maximal power; we used a method by Falconer, taking allele frequency and mean nonfasting glucose levels for each genotype into account (19).

Third, to test whether genetically elevated nonfasting glucose levels associate with increased risk of IHD, we tested for association between genotype and IHD and MI risk using logistic regression in the CCHS, CGPS, and CIHDS combined to obtain maximal power. Analyses were adjusted for age and sex only, as genotypes were randomly distributed across the covariates.

Finally, instrumental variable analysis by 2-stage least-squares regression was used to assess a potential causal relationship between elevated nonfasting glucose levels and increased IHD and MI risk using genetic variants as instruments for elevated nonfasting glucose levels in an additive model; we used the CCHS, CGPS, and CIHDS combined to obtain maximal power. Strength of the genotypes as instruments (association of genotype with plasma glucose) was evaluated by F-statistics from the first-stage regression, where $F > 10$ indicates sufficient strength to ensure the validity of the instrumental variable analysis, while R^2 in percent is a measure of percent contribution of genotype to the variation in plasma glucose (4). Causal odds ratios were estimated using the multiplicative generalized method of moments estimator implemented in the user-written Stata command "ivpois." Altman's method (20) was used to compare the causal odds ratio from the instrumental variable analysis with the observational multifactorially adjusted hazard ratio from Cox regression. Use of >1 genotype as instrumental variable reduces risk of bias

Table 1 Characteristics of Participants

Characteristics	CCHS	CGPS	CIHDS
n	16,568	48,614	15,340
Ischemic heart disease*	4,184	4,862	5,109
Myocardial infarction*	2,327	2,038	1,892
Age, yrs	51 (40–59)	58 (47–67)	57 (48–66)
Women	53%	56%	46%
Body mass index, kg/m^2	24.3 (22.2–26.8)	25.6 (23.2–28.5)	25.6 (23.2–28.5)
Total cholesterol, mmol/l	6.0 (5.2–6.8)	5.6 (4.9–6.3)	5.5 (4.7–6.3)
HDL cholesterol, mmol/l	1.4 (1.1–1.7)	1.6 (1.3–2.0)	1.5 (1.2–1.9)
Diabetes mellitus	2.1%	3.7%	13.2%
Hypertension	47%	71%	55%
Current smoking	53%	20%	26%
Menopause, women only	49%	66%	—
Statin use	1.5%	11%	—

Values are n, mean (range), or %. *Number of disease events at end of follow-up. Other data are from enrollment into the studies: In the CCHS (Copenhagen City Heart Study), values are from the first participation in the study in either 1976 to 1978, 1981 to 1983, 1991 to 1994, or 2001 to 2003; in the CGPS (Copenhagen General Population Study), from enrollment 2003 to 2010; and in the CIHDS (Copenhagen Ischemic Heart Disease Study), from enrollment 1994 to 2010. To convert glucose in mmol/l to mg/dl, multiply by 18; to convert cholesterol in mmol/l to mg/dl, multiply by 39.

HDL = high-density lipoprotein.

due to pleiotropy, but may result in an overestimation of the causal risk estimate (21). This can be evaluated using effect estimates (strength of the association between genotype and glucose level) from previous independent studies. To do this, we repeated the instrumental variable analyses using effect estimates from published genome-wide association studies (effect sizes shown in Fig. 2) (5,6,8).

Results

Characteristics of the 80,522 participants in the 3 study populations are shown in Table 1; IHD developed in 14,155 subjects and MI in 6,247. Age, BMI, total cholesterol levels, frequency of male sex, metabolic syndrome, diabetes mellitus, use of insulin or oral antidiabetic medication, hypertension, smoking, menopause (women only), and statin use were higher at higher nonfasting plasma glucose levels, whereas HDL cholesterol levels were lower (Online Table 1). Variation in nonfasting plasma glucose levels as a function of time since the last meal in hours is shown in Online Figure 1. Median values ranged from 5.66 mmol/l at 0 to 1 h after a meal to 5.08 mmol/l >8 h after a meal (range 0.58 mmol/). Regression dilution on plasma glucose levels from the 1976 to 1978 to the 1981 to 1983 examination is shown in Online Figure 2, and was used to correct association between nonfasting glucose and risk of IHD and MI for regression dilution bias.

Nonfasting glucose and IHD and MI observational estimates. Risk of IHD and MI increased stepwise with increasing nonfasting glucose levels (Figs. 1 and 2). Subjects with a level >11 mmol/l had their event on average 20 years before subjects with levels <5 mmol/l (Fig. 1). Age- and sex-adjusted hazard ratio for IHD was 6.9 (95% confidence interval [CI]: 4.2 to 11.2) in subjects with nonfasting plasma glucose levels ≥11 mmol/l (≥198 mg/dl) versus <5 mmol/l (<90 mg/dl). Corresponding estimates were 6.2 (95% CI: 3.8 to 10.2) when adjusting multifactorially; 4.7 (95% CI: 2.8 to 7.7) multifactorially including BMI; 3.8 (95% CI: 2.2 to 6.5) multifactorially including total and HDL cholesterol; 5.8 (95% CI: 3.6 to 9.5) multifactorially including hypertension; and 2.3 (95% CI: 1.3 to 4.2) multifactorially including all the above. Corresponding values for MI were 9.2 (95% CI: 4.6 to 18.2), 8.3 (95% CI: 4.2 to 16.6), 6.8 (95% CI: 3.4 to 13.7), 6.0 (95% CI: 2.8 to 13.0), 7.5 (95% CI: 3.8 to 15.0), and 4.8 (95% CI: 2.1 to 11.2).

Genotype and nonfasting glucose. Homozygotes versus noncarriers associated with the following increases in nonfasting plasma glucose levels: *GCK* (rs4607517) 2.7% (95% CI: 1.5% to 3.8%), *G6PC2* (rs560887) 2.5% (1.9% to 3.2%), *ADCY5* (rs11708067) 1.5% (0.7% to 2.2%), *DGKB* (rs2191349) 0.8% (0.3% to 1.3%), and *ADRA2A* (rs10885122) 0.5% (−1.1% to 2.0%) (Fig. 3). Combining genotypes by number of glucose-increasing alleles, an increasing number of glucose-increasing alleles associated with an up to 4.8% (3.6% to 6.0%) increase in nonfasting glucose levels.

Genotype and IHD and MI. Assuming that increased nonfasting glucose levels have a causal effect on risk of IHD and MI, lifelong increased glucose levels due to genetic variants should confer a similar increase in risk of IHD and MI as that observed for increased glucose levels encountered in the general population. For example, the 4.8% increase in nonfasting glucose levels seen for 8 glucose-increasing alleles (Fig. 3) would theoretically predict an increased risk of IHD and MI with hazard ratios of 1.04 (95% CI: 1.03 to 1.05) and 1.04 (95% CI: 1.03 to 1.05) (Online Fig. 3). In accordance with this, the observed odds ratio for IHD increased as a function of the glucose-increasing alleles with the largest glucose-increasing effects (p trend = 0.01) (Online Fig. 3); the

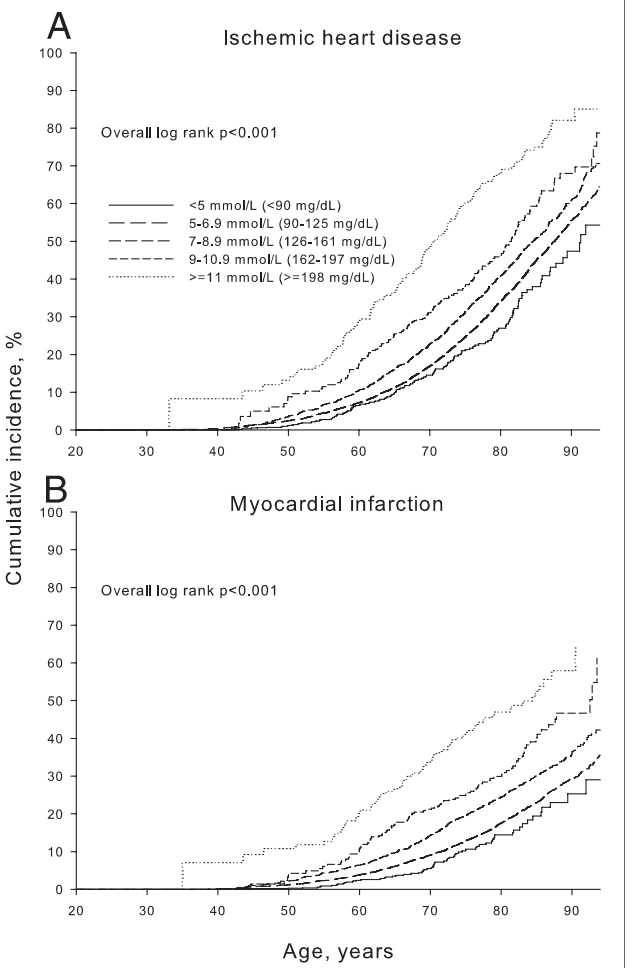

Figure 1 **Cumulative Incidence Using Kaplan-Meier Estimates of IHD and MI**

Cumulative incidence using Kaplan-Meier estimates of **(A)** ischemic heart disease (IHD) and **(B)** myocardial infarction (MI) in percent as a function of age at event. Subjects with IHD or MI before study enrollment were excluded, resulting in 16,318 subjects followed up for as long as 35 years with respect to incident events. **Solid line** indicates <5 mmol/l; **long-dashed line** indicates 5 to 6.9 mmol/l; **shorter-dashed line** indicates 7 to 8.9 mmol/l; **shortest-dashed line** indicates 9 to 10.9 mmol/l; and **dotted line** indicates ≥11 mmol/l.

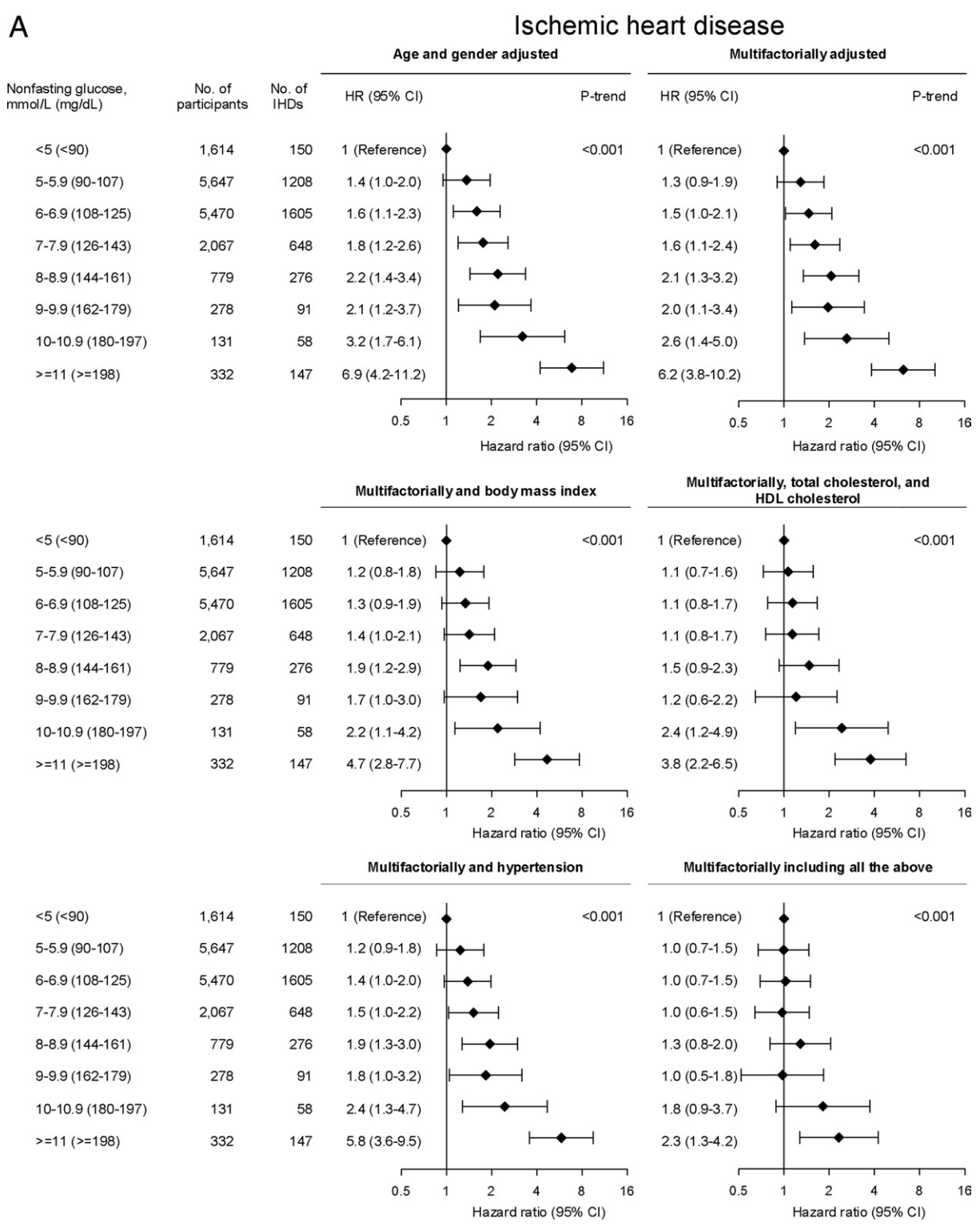

Figure 2 **Risk of IHD and MI as a Function of Baseline Nonfasting Plasma Glucose Levels in General Population**

Nonfasting plasma glucose levels were measured in 16,568 subjects who participated in the 1976 to 1978, 1981 to 1983, 1991 to 1994, and/or 2001 to 2003 examinations of the Copenhagen City Heart Study. Results are shown for **(A)** ischemic heart disease (IHD) and **(B)** myocardial infarction (MI). Participants with IHD or MI before study enrollment were excluded, resulting in 16,318 subjects followed up for as long as 35 years with respect to incident events. Basic multifactorial adjustment was for age, sex, current smoking, menopause, and statin use. The fully multifactorially adjusted model additionally included body mass index, total cholesterol, high-density lipoprotein (HDL) cholesterol, and hypertension. CI = confidence interval; HR = hazard ratio.

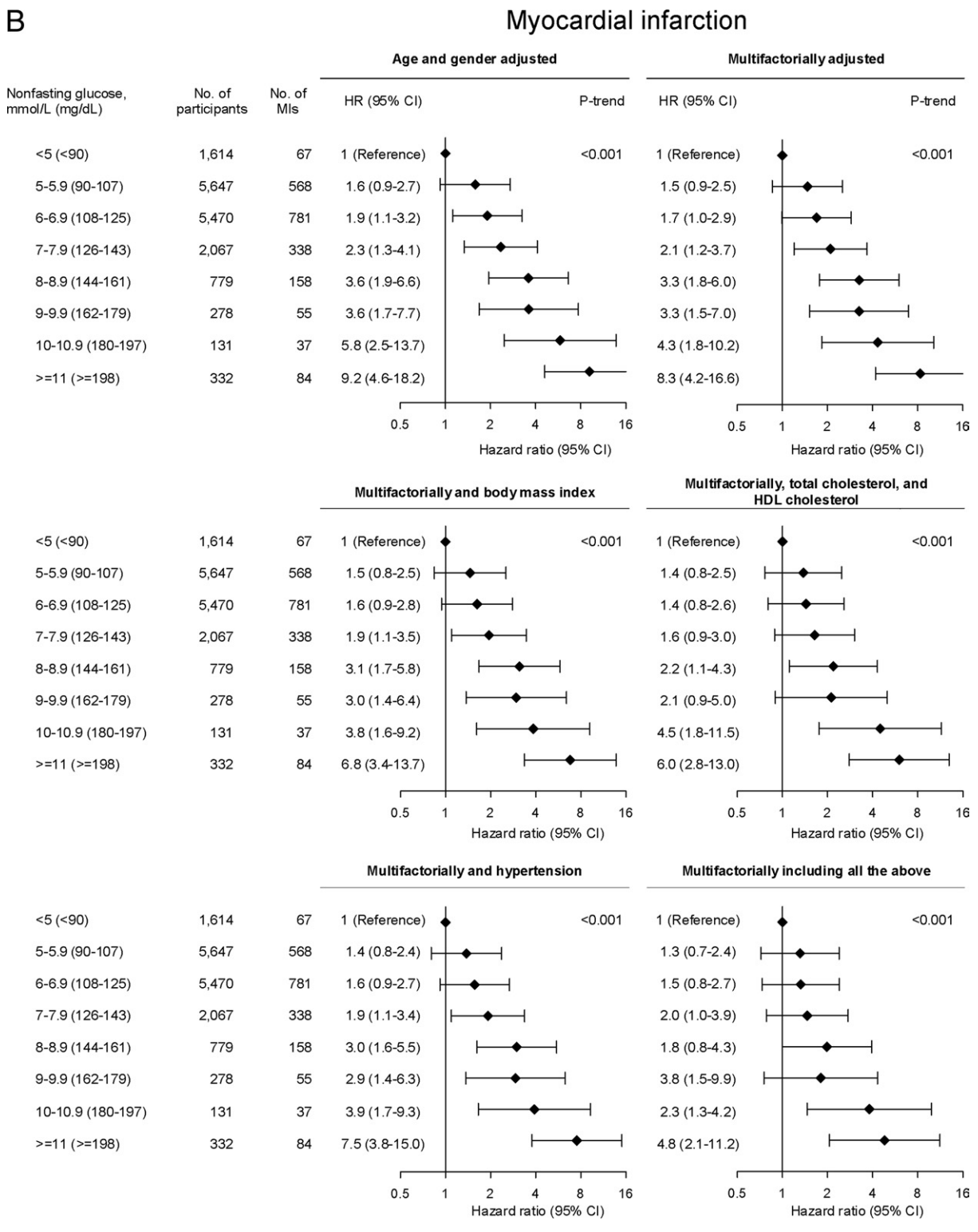

Figure 2 Continued

various risk factors except glucose levels were equally distributed among the genotypes, confirming that these genotypes are largely unconfounded by such factors (Online Table 2). Results for MI were similar (Online Fig. 3).

Nonfasting glucose and IHD and MI causal estimates. A potential causal effect of increased nonfasting glucose levels on increased risk of IHD and MI was also examined using instrumental variable analysis. A 1-mmol/l (18-mg/

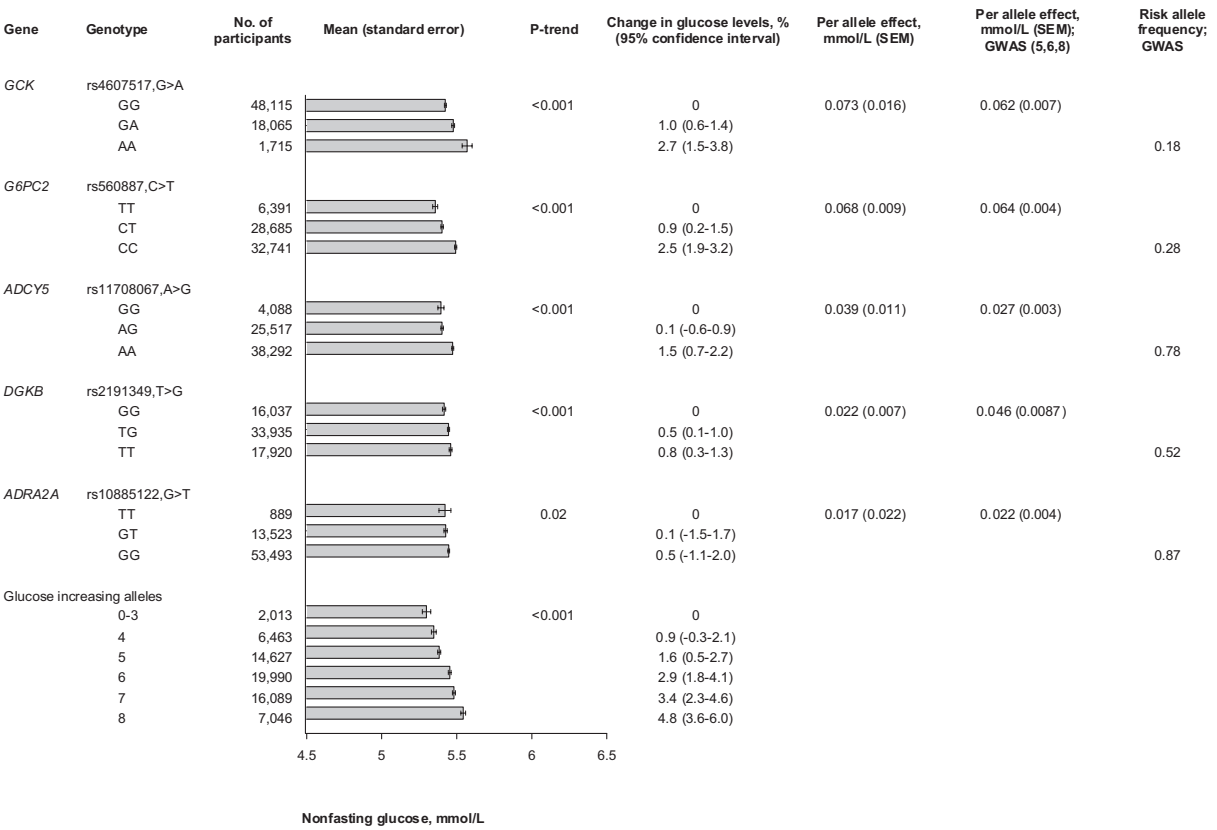

Figure 3 Nonfasting Plasma Glucose Levels as a Function of Genotype in General Population

Glucose-increasing alleles were combinations of genotypes with glucose-increasing effects; the most common genotype combinations are shown, whereas the rarer combinations were excluded because of reduced statistical power. This was examined in 67,914 participants from the Copenhagen City Heart Study and the Copenhagen General Population Study combined, irrespective of other cardiovascular risk factors or treatment for diabetes mellitus. Estimates of effect on fasting plasma glucose levels reported from genome-wide association studies (GWAS) using plasma glucose level as the phenotype are reported in the right column. To convert mmol/l glucose to mg/dl, multiply by 18.

dl) increase in nonfasting glucose levels due to genotypes associated with a causal odds ratio for IHD of 1.25 (95% CI: 1.03 to 1.52) and MI of 1.69 (95% CI: 1.28 to 2.23), and the observed multifactorially adjusted hazard ratio for a similar increase was 1.18 (95% CI: 1.15 to 1.22) for IHD and 1.09 (95% CI: 1.07 to 1.11) for MI (Fig. 4). The corresponding causal odds ratios for IHD and MI were 1.36 (95% CI: 1.05 to 1.77) and 1.54 (95% CI: 1.05 to 2.27), respectively, using effect estimates for per allele increases in fasting glucose levels from the literature.

Discussion

The main finding of this study is that both observational and genetically elevated nonfasting glucose levels are associated with increased risk of IHD and MI. This finding is compatible with elevated glucose levels per se being causally related to the development of IHD and MI.

Overall, evidence for or against a causal contribution of glucose to the pathogenesis of macrovascular disease, including IHD and MI, can come from 5 types of evidence; that is, conventional epidemiology, mechanistic studies, animal models, randomized intervention trials, and Mendelian randomization studies like the present (22,23). 1) Previous prospective epidemiologic studies showed that elevated fasting glucose levels associate with increased IHD and MI risk even at nondiabetic glucose levels (1,2). Our results using nonfasting glucose levels are in agreement with this, and confirm that even after adjustment for obesity, dyslipidemia, and hypertension, elevated IHD and MI risk remain. 2) Results from in vitro and animal studies have suggested several mechanisms by which glucose may contribute to macrovascular disease: increased glucose levels, free fatty acids, and insulin resistance together leads to oxidative stress, activation of protein kinase C isoforms, formation of advanced glycation end product, and nonenzymatic glycation of low-density lipoprotein, apolipoproteins, and clotting factors, collectively resulting in vasoconstriction, inflammation, and thrombosis (2,24,25). 3) In animal models with experimental hypercholesterolemia or genetic predisposition for atherosclerosis, elevated glucose

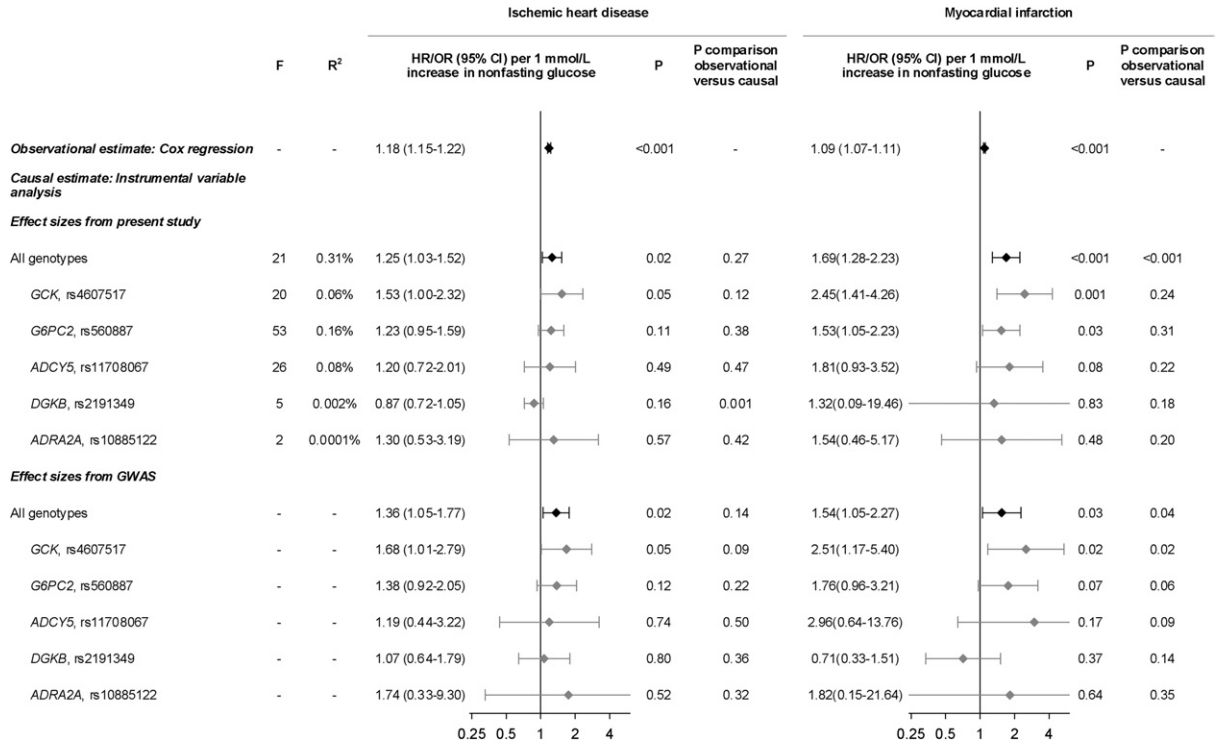

Figure 4 **Summary of Causal Effects of Increased Nonfasting Glucose on Increased Risk of IHD and MI**

Studies' summary of the causal effect of increased nonfasting glucose on increased risk of ischemic heart disease (IHD) and myocardial infarction (MI). This was tested in the Copenhagen City Heart Study, the Copenhagen General Population Study, and the Copenhagen Ischemic Heart Disease Study combined; 6,448 participants were not genotyped for lack of deoxyribonucleic acid; and for participants genotyped, numbers vary slightly from genotype to genotype. The causal effect of increased nonfasting glucose levels on risk of IHD and MI was estimated by the association between a 1-mmol/l (18-mg/dl) genetic increase in nonfasting glucose levels and risk of IHD and MI, using instrumental variable analysis by 2-stage least-squares regression and given as an odds ratio (OR) with a 95% confidence interval (CI). This risk is compared to the observational prospective increased risk of IHD and MI, respectively, associated with a 1-mmol/l (18-mg/dl) increase in nonfasting glucose in the general population, the Copenhagen City Heart Study, given as a multifactorially adjusted Cox regression hazard ratio (HR) with a 95% CI. Odds ratios for a 1-mmol/l (18-mg/dl) genetic increase in nonfasting glucose levels are also given for individual and combined genotypes. F-statistics (evaluation of strength of instrument) and R^2 (contribution of genotype to variation in plasma glucose levels in percent) are from the first-stage regression analysis. In the second-stage regression analysis studying the association between genotype and risk, imputed glucose values were included in part of the cases in the Copenhagen Ischemic Heart Disease Study. The causal effect of a 1-mmol/l (18-mg/dl) genetic increase in nonfasting glucose levels on risk of IHD and MI was also estimated using effect sizes for genotypes previously reported from genome-wide association studies specifically aimed at identifying genes associated with plasma glucose levels. The p value is for significance of hazard ratio/odds ratio. The p value comparison is between the estimate from observational epidemiology and the causal estimate from instrumental variable analysis. To convert mmol/l glucose to mg/dl, multiply by 18.

levels may directly cause macrovascular disease (24,26). 4) A meta-analysis of randomized intervention trials (27–32) showed that intensive glycemic control in patients with diabetes mellitus was associated with a 15% reduction in risk of IHD (3). However, in all included studies, intensive glycemic control also had beneficial effects on obesity, dyslipidemia, and/or hypertension, obscuring the isolated effect of reduced glucose levels, and this issue remains unresolved. 5) Using a Mendelian randomization approach free from reverse causation and unconfounded by obesity, dyslipidemia, and hypertension, we here found that both observational and genetic lifelong elevated nonfasting or fasting glucose levels associate with increased risk of IHD and MI. In the present study, we use genotypes associated with glucose levels in the nondiabetic range and not associated with diabetes mellitus in our general population samples, suggesting that the increased risk of IHD is more likely due to glucose per se, rather than mediated through diabetes mellitus. A recent Mendelian randomization study support that elevated fasting plasma glucose levels may also be causally related to increased intima-media thickness (33). Therefore, these 5 different types of evidence collectively suggest that elevated plasma glucose per se might be causally related to the development of IHD and MI.

From a clinical standpoint, our confirmation of increased nonfasting glucose as a marker of increased IHD risk suggests that nonfasting values may be used in risk stratification, whereas for diagnostic purposes, fasting values are required. Although nonfasting/post-prandial glucose levels are more variable than fasting levels, risk of cardiovascular disease is more strongly associated with nonfasting/post-prandial hyperglycemia than fasting hyperglycemia (34). The exact reason for this is not known, but it has been observed that persons with impaired glucose tolerance, a

diagnostic group known to have a high risk of cardiovascular disease, have elevated nonfasting/post-prandial glucose levels, but usually fasting blood glucose levels within the normal range (35). The use of nonfasting glucose measurements may explain the relatively high observational risk estimates in the present study compared to others (1), but this difference may also in part be because we were able to adjust for regression dilution bias (18).

Strengths of the present study are that we had sufficient statistical power to document increased risk of IHD and MI as a function of glucose-increasing genotypes. Another strength of the present study was that all participants were white persons of Danish descent, thus effectively excluding admixture as a potential problem in our study.

A potential limitation of the present Mendelian randomization approach is that, apart from their involvement in glucose regulation and metabolism, the presently studied genetic variants may have pleiotropic effects on other cardiovascular risk factors or directly on IHD and MI risk unrelated to nonfasting glucose levels. However, we did not find any consistent associations with age, BMI, levels of total cholesterol or HDL cholesterol, or frequency of metabolic syndrome, diabetes mellitus, use of insulin or oral antidiabetic medication, hypertension, current smoking, menopause, or statin use across genotypes. Nevertheless, the *ADCY5* and *DGKB* genes are reported to be involved in symphacetic and parasymphacetic regulation of heart rate (10) and may have an effect on IHD and MI risk through 1 of these pathways. Furthermore, *ADRA2A* is directly involved in lipolysis and is associated with risk of attention deficit/hyperactive disorder (11). Except for increased birth weight reported to be associated with *GCK* genotype, no pleiotropic effects have been reported for the *GCK* and *G6PC2* genes with the largest effects on nonfasting glucose levels in the present study. Using >1 genotype reduces the effect of unknown pleiotropy of the genotypes and reduces the influence of a genotype directly associated with risk of IHD and MI unrelated to nonfasting glucose levels, but may result in an overestimation of the causal risk (4). Another potential limitation of the present study is the modest contribution of genotypes to the variation in nonfasting glucose levels (weak instrument bias and unreliable instruments bias) and the use of 5 genotypes as instruments. To evaluate this, we first used F statistics to test the strength of the instruments and found that 3 of 5 instruments were excellent, and combined, all 5 genotypes were good instruments ($F > 10$). We then repeated the instrumental variable analyses using effect estimates from published genome-wide association studies. The causal estimate using external independent effect sizes corresponded with the causal estimate from the present study populations, suggesting that such a bias is unlikely. Also, we studied white subjects only, and therefore our results may not necessarily translate to other races. A known limitation of prospective studies using a baseline nonfasting measurement for classification is misclassification due to regression dilution bias, which is the fact that extreme values at baseline tend to attenuate over time and regress toward the mean value of measurements (4). In the present study, we corrected for this using results from subjects with >1 measurement over time to estimate the degree of regression dilution.

Conclusions

Both elevated observational and genetic nonfasting glucose levels are associated with increased risk of IHD and MI. These data are compatible with a causal association.

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Key Words: cardiovascular disease ■ diabetes mellitus ■ epidemiology ■ nonfasting ■ plasma glucose ■ post-prandial.

APPENDIX

For supplemental figures and tables, please see the online version of this article.