A genetic risk score for fasting plasma glucose is independently associated with arterial stiffness: a Mendelian randomization study

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Background: Arterial stiffness is known to be associated with a number of clinical conditions including hypertension, diabetes and dyslipidemia, and may predict cardiovascular events and mortality. However, causal links are hard to establish. Results from genome-wide association studies have identified only a few single nucleotide polymorphisms associated with arterial stiffness, the results have been inconsistent between studies and overlap with other clinical conditions is lacking. Our aim was to investigate a potential shared set of risk single nucleotide polymorphisms between relevant cardiometabolic traits and arterial stiffness.

Method: The study population consisted of 2853 individuals (mean age 72 years, 40% men) from the population-based Malmö Diet and Cancer study, Sweden. Carotid–femoral pulse wave velocity, a marker of arterial stiffness, was measured with Sphygmocor. Mendelian randomization analyses were performed using the two-stage least square regression and multivariate inverse-variance weighted methods.

Results: There were positive associations between arterial stiffness and genetic risk scores for type 2 diabetes ($\beta = 0.03$, $P = 0.04$) and fasting plasma glucose ($\beta = 0.03$, $P = 0.03$), but not for systolic blood pressure, body mass index, low-density lipoprotein cholesterol, high-density lipoprotein cholesterol or triglycerides. Multivariate inverse-variance weighted methods confirmed the significant positive association for fasting plasma glucose β coefficients ($P = 0.006$), but not for type 2 diabetes β coefficients ($P = 0.88$).

Conclusion: Genetically elevated fasting plasma glucose, but not genetically elevated risk of type 2 diabetes, was associated with arterial stiffness suggesting a causal stiffening effect of glycemia on the arterial wall, independently of type 2 diabetes.

Keywords: aging, arterial stiffness, diabetes mellitus, epidemiology, hyperglycemia, Mendelian randomization

Abbreviations: c-f PWV, carotid–femoral pulse wave velocity; FPG, fasting plasma glucose; GRSs, genetic risk scores; GWAS, genome-wide association study; HDLc, high-density lipoprotein cholesterol; LDLc, low-density lipoprotein cholesterol; SNP, single nucleotide polymorphism; T2D, type 2 diabetes

INTRODUCTION

The stiffening of large elastic arteries that occurs with increasing age is an important contributor to hypertension, especially in the elderly [1,2]. Arterial stiffness has also been shown to be an independent predictor of cardiovascular events and mortality [3,4]. In addition to the strong inter-correlation of arterial stiffness with blood pressure, several cross-sectional studies have shown arterial stiffness to be associated with a number of clinical conditions such as diabetes, dyslipidemia and adiposity [5–7]. Markers of these conditions have been able to predict arterial stiffness after a long follow-up time. However, observational studies of this kind cannot prove causality.

Despite associations of arterial stiffness with several cardiovascular risk markers, genome-wide association studies (GWAS) have identified only one genetic variant to be associated with arterial stiffness and results have not been concurrent [8,9]. This significant locus in the 3'-*BCL11B* gene desert on chromosome 14 was identified in a multicenter GWAS in 20 364 individuals from nine European community-based cohorts [9].

Mendelian randomization is an analysis technique, which uses genetic information to achieve an unbiased detection of causal effects [10]. Benefitting from the random allocation of alleles at conception and the absence of confounding factors or reverse causality, in Mendelian randomization, the genetic variant is used as an instrumental variable [11].

The aim of this study was to use a Mendelian randomization approach to explore a potential causal relationship between arterial stiffness and cardiometabolic risk factors by the use of genetic risk score (GRS) of blood pressure, adiposity, disturbed glucose metabolism and dyslipidemia.

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METHODS

Study population

The study population is part of the Malmö Diet and Cancer (MDC) study, a large population-based cohort consisting of men and women from the city of Malmö, Sweden [12,13]. A subset of the population, 6103 individuals, took part in the cardiovascular arm of this cohort investigated in 1991–1994 [14]. Of these original 6103 participants, 3734 individuals were re-investigated in 2007–2012, of which 3056 individuals underwent measurement of pulse wave velocity (PWV). This has been previously described including analysis of nonparticipation at the re-investigation [15] and PWV measurements [5]. The study was approved by the regional ethics review board, Lund, Sweden (ID 532-2006). A written informed consent was obtained from all participants.

Altogether, there is complete data on 2853 individuals constituting the study population for our analyses.

Measurement of arterial stiffness

Arterial stiffness was measured as carotid–femoral pulse wave velocity (c-f PWV) using an applanation tonometry technique (SphygmoCor, Atcor Medical, Australia) with participants in a quiet room in supine position after 5 min of rest. The carotid–femoral distance was calculated using the so-called subtraction method defining the distance as the suprasternal notch to the umbilicus plus the umbilicus to the measuring point at the femoral artery minus the suprasternal notch to the measuring point at the carotid artery. Transit time was calculated using the foot-to-foot method with simultaneous ECG registration measuring the time from the peak of the R-wave on the ECG to the foot of the pulse wave at the carotid and femoral arteries, respectively. The number of successful c-f PWV measurements in each individual varied from one to five, with a goal of three measurements (86.7% of cases). Results are based on mean values from these measurements.

Genotyping

The single nucleotide polymorphisms (SNPs) were genotyped using a MALDI-TOF mass spectrometer (Sequenom Mass Array, Sequenom, San Diego, California, USA). Proxy SNPs were found using SNAP version 2.2. SNPs that failed this analysis were analyzed individually using the Taqman or KASPar allelic discrimination method on an ABI 790HT (Applied Biosystems, Life Technologies, Carlsbad, California, USA), according to instructions provided by the manufacturer. Individuals with less than 60% successful genotyped SNPs were excluded. SNPs with a genotype success rate of less than 90% or deviation from the Bonferroni-corrected Hardy–Weinberg Equilibrium in each set of SNPs for each trait were excluded from the analysis. At least 25% of individuals were also genotyped with a different method (Human OmniExpress Bead Chip; Illumina, San Diego, California, USA) to check for concordance, which was more than 98% for all included SNPs.

Construction of genetic risk scores

Construction of weighted GRS for SBP, BMI, low-density lipoprotein cholesterol (LDLc), high-density lipoprotein

cholesterol (HDLc), triglycerides, fasting plasma glucose (FPG) and type 2 diabetes (T2D) were performed using publications from large multicenter genome-wide association studies (GWASs) [16–24]. The SNPs included in the GRSs were weighted by the reported effect size in GWAS. The SNPs for FPG were all discovered in nondiabetic individuals [16]. Of the 15 SNPs included in the FPG GRS, 7 overlapped with the SNPs in the T2D GRS. A GRS for T2D without the seven SNPs overlapping in the FPG GRS was created. This GRS is referred to as T2D41 GRS (as it includes 41 SNPs). Also for the other GRSs, a few SNPs had shown GWAS significance for several traits and, thus, were included in several scores. The references and number of included SNPs for the respective GRSs are presented in Supplementary Table 1, <http://links.lww.com/HJH/A883> and complete tables of all included SNPs are presented in Supplementary Tables 2–8, <http://links.lww.com/HJH/A883>. The genotype at each locus was coded as 0, 1 or 2 depending on the number of alleles, previously shown to increase the risk factor in question. With information from previous publications, each allele was weighted according to the estimated effect size. We both calculated a GRS for each individual and used a multivariate Mendelian randomization wherever causal estimates calculated from each SNP were combined.

Statistical analysis

Statistical calculations were performed using IBM SPSS Statistics, version 22 (IBM Corp., Armonk, New York, USA), PLINK (version 1.07) and R (version 3.31). C-f PWV, LDLc, HDLc, triglycerides and FPG were natural logarithm transformed to achieve normal distributions.

The associations between each GRS and its respective trait as well as the associations between each trait and c-f PWV were calculated with multiple linear regression.

For the GRSs calculated for each individual, multiple linear regression was used to investigate the association between the respective GRS and c-f PWV with adjustment for age, sex and mean arterial pressure (MAP). Logistic regression was used to calculate the odds ratio of GRS for T2D. Whenever the instrumental variable was SBP GRS, ongoing blood pressure-lowering treatment was added to the analysis and additionally, sensitivity analyses without antihypertensive medication was performed. Whenever the instrumental variable was LDLc GRS, HDLc GRS or triglycerides GRS, lipid-lowering treatment was added to the analysis and, sensitivity analysis without individuals on statin treatment was performed in addition to the main analyses. Whenever the instrumental variable was FPG GRS, individuals with diabetes were excluded. To estimate the causal effect of the trait on c-f PWV, we performed a two-stage least squares regression [10]. Firstly, the traits were regressed on their respective GRS and the fitted values were saved. Secondly, c-f PWV was regressed on the predicted fitted values. Adjustments were made for age, sex and MAP in both stages.

Also, an inverse-variance weighted Mendelian randomization regression was performed according to a previously described approach [25,26]. This was performed as a complementary approach, in order to correct for potential bias

TABLE 1. Characteristics of the study population, $n=2853$

Characteristics	Included individuals ($n=2853$)	Excluded individuals ($n=203$)	
	Mean ($\pm$ SD)	Mean ($\pm$ SD)	P
Age (years)	72.1 (5.5)	71.8 (5.5)	0.50
SBP (mmHg)	136 (17)	137 (18)	0.34
DBP (mmHg)	76 (9)	76 (10)	0.98
BMI (kg/m^2)	26.7 (4.2)	26.6 (4.4)	0.74
LDLc (mmol/l)	3.3 (0.9)	3.4 (1.1)	0.26
HDLc (mmol/l)	1.4 (0.4)	1.4 (0.5)	0.38
GRS BMI	0.06 (0.008)	—	—
GRS SBP	0.58 (0.07)	—	—
GRS LDLc	1.12 (0.13)	—	—
GRS HDLc	−0.37 (0.04)	—	—
GRS triglycerides	2.53 (0.30)	—	—
GRS FPG	0.02 (0.003)	—	—
GRS T2D	0.60 (0.05)	—	—
GRS T2D41	0.60 (0.05)	—	—

	Median (first to third quartile)	Median (first to third quartile)	
Triglycerides (mmol/l)	1.0 (0.7–1.3)	1.0 (0.8–1.5)	0.05
FPG (mmol/l)	5.8 (5.4–6.4)	5.9 (5.3–6.5)	0.57
C-f PWV (m/s)	10.1 (8.8–11.8)	10.2 (8.9–12.0)	0.60

	n (%)	n (%)	
Sex (percentage men)	1147 (40.2)	61 (30.0)	0.004
Diabetes	401 (14.1)	37 (18.2)	0.10
Blood pressure-lowering therapy	1539 (53.9)	116 (57.1)	0.38
Lipid-lowering drug therapy	826 (29.0)	69 (34.0)	0.13

c-f PWV, carotid–femoral pulse wave velocity; FPG, fasting plasma glucose; GRS, genetic risk score; HDLc, high-density lipoprotein cholesterol; LDLc, low-density lipoprotein cholesterol; T2D, type 2 diabetes; T2D41, T2D GRS without the seven single nucleotide polymorphisms overlapping in the FPG GRS.

of pleiotropic effects of a SNP on any of the other studied traits. Shortly, the β coefficients from the multiple linear regression of each of the 183 SNPs on c-f PWV were regressed on the β coefficients from the multiple linear regression of the same SNPs on each trait. This regression was inverse-variance weighted using standard errors of each SNP–PWV association and the intercept was fixed to zero.

RESULTS

The characteristics of the final study population of 2853 individuals are presented in Table 1. The population with c-f PWV data but without a complete data set ($n=203$) had a higher proportion of women (70.0 versus 59.8%, $P=0.004$),

otherwise there were no significant differences between the study population and individuals excluded because of missing data.

All traits were associated with c-f PWV as presented in Table 2. Each GRS, except for SBP GRS, was significantly associated with its respective trait. This is presented in Table 3.

There were significant associations between c-f PWV and both FPG GRS ($\beta=0.03$, $P=0.03$) and T2D GRS ($\beta=0.03$, $P=0.04$). There was no significant association between T2D41 GRS and c-f PWV ($\beta=0.03$, $P=0.07$). In sensitivity analyses without individuals with diabetes the significance for FPG GRS remained ($\beta=0.04$, $P=0.02$).

TABLE 2. Association of traits with carotid–femoral pulse wave velocity, adjusted for age and sex

Trait	β	P
BMI ^a	0.11	2×10^{-12}
SBP ^c	0.38	1×10^{-121}
LDLc ^{a,b}	−0.04	0.04
HDLc ^{a,b}	−0.11	6×10^{-11}
Triglycerides ^{a,b}	0.13	5×10^{-17}
FPG ^a	0.16	7×10^{-23}
Diabetes ^a	0.16	9×10^{-25}

Associations calculated with multiple linear regressions. FPG, fasting plasma glucose; HDLc, high-density lipoprotein cholesterol; LDLc, low-density lipoprotein cholesterol.

^aFurther adjusted for mean arterial pressure.

^bFurther adjusted for lipid-lowering treatment.

^cFurther adjusted for blood pressure-lowering treatment.

TABLE 3. Association of genetic risk scores with its respective trait, measured at the Malmö Diet and Cancer follow-up examination

GRS	β	P
BMI	0.063	0.001
SBP ^a	0.016	0.40
LDLc ^b	0.24	6×10^{-28}
HDLc ^b	0.21	1×10^{-28}
Triglycerides ^b	0.20	4×10^{-28}
FPG ^c	0.14	3×10^{-12}
T2D	0.11	4×10^{-9}
T2D41	0.09	2×10^{-6}

Associations calculated with multiple linear regressions. FPG, fasting plasma glucose; GRS, genetic risk score; HDLc, high-density lipoprotein cholesterol; LDLc, low-density lipoprotein cholesterol; T2D, type 2 diabetes; T2D41, T2D GRS without the seven single nucleotide polymorphisms overlapping in the FPG GRS.

^aAdjusted for blood pressure-lowering treatment.

^bAdjusted for lipid-lowering treatment.

^cExcluding individuals with diabetes at follow-up.

TABLE 4. Association between genetic risk scores and carotid–femoral pulse wave velocity adjusted for age, sex, mean arterial pressure and ongoing blood pressure-lowering treatment

GRS	β_{PWV}^a	P^a	β_{PWV}^b	P^b
BMI	0.01	0.70	Not applicable	Not applicable
SBP	−0.03	0.05	−0.04	0.10
LDLc	−0.004	0.78	0.02	0.42
HDLc	−0.02	0.13	−0.02	0.24
Triglycerides	0.03	0.05	0.01	0.56
FPG	0.03	0.03	0.04	0.02
T2D	0.03	0.04	Not applicable	Not applicable
T2D41	0.03	0.07	Not applicable	Not applicable

Associations calculated with multiple linear regressions. GRS, genetic risk score; LDLc, low-density lipoprotein cholesterol; HDLc, high-density lipoprotein cholesterol; FPG, fasting plasma glucose; T2D, type 2 diabetes; T2D41, T2D GRS without the seven single nucleotide polymorphisms overlapping in the FPG GRS.

^a β for LDL, HDL and triglycerides also adjusted for lipid-lowering treatment.

^bSensitivity analyses excluding individuals on lipid lowering medication in the lipid analyses, blood pressure lowering treatment in the blood pressure analyses and diabetes in the FPG analysis.

There were borderline significant associations for SBP GRS ($\beta = -0.03$, $P = 0.05$) and triglycerides GRS ($\beta = 0.03$, $P = 0.05$), but none of these remained in sensitivity analyses excluding individuals with blood pressure-lowering treatment and lipid-lowering treatment, respectively. For the other GRSs there were no significant associations with c-f PWV (see Table 4).

In two-stage least squares regression, the instrumental variable, one standard deviation (SD) increment of FPG GRS, was associated with 0.034 SD (95% CI 0.003–0.065) or 0.08 m/s (95% CI 0.007–0.16) higher c-f PWV.

A total of 183 unique SNPs ($r^2 < 0.2$) that were reported in GWAS to associate with SBP, BMI, LDLc, HDLc, triglycerides, FPG and T2D were included in the inverse-variance weighted Mendelian randomization. There was a significant association between c-f PWV β coefficients and FPG β coefficients ($\beta = 0.28$, $P = 0.006$), but not with T2D β coefficients ($\beta = 0.006$, $P = 0.88$). There were no significant associations between c-f PWV β coefficients and BMI ($\beta = -0.03$, $P = 0.82$), SBP ($\beta = 0.002$, $P = 0.98$), LDLc ($\beta = -0.05$, $P = 0.32$), HDLc ($\beta = -0.02$, $P = 0.73$) or triglyceride ($\beta = 0.09$, $P = 0.18$) β coefficients. This is presented in Table 5. When excluding eight SNPs were associated with FPG but not with T2D, FPG β coefficients were still significantly associated with PWV β coefficients ($\beta = 0.25$, $P = 0.018$) and T2D results remained insignificant ($\beta = 0.04$, $P = 0.33$).

TABLE 5. Inverse-variance weighted Mendelian randomization of cardiometabolic traits and carotid–femoral pulse wave velocity adjusted for age, sex, mean arterial pressure and blood pressure lowering treatment at follow-up

	β_{PWV}^a	P	β_{PWV}^b	P
β_{BMI}	−0.03	0.82	−0.03	0.80
β_{SBP}	0.002	0.98	0.02	0.91
β_{LDLc}	−0.05	0.32	−0.06	0.27
β_{HDLc}	−0.02	0.73	−0.02	0.75
$\beta_{triglycerides}$	0.09	0.18	0.08	0.16
β_{FPG}	0.28	0.006	0.28	0.006
β_{T2D}	0.006	0.88	0.006	0.88

FPG, fasting plasma glucose; HDLc, high-density lipoprotein cholesterol; LDLc, low-density lipoprotein cholesterol; PWV, pulse wave velocity; T2D, type 2 diabetes.

^a β_{SBP} obtained after adjustment for age, sex, and antihypertensive treatment at baseline. β_{LDLc} , β_{HDLc} and β_{TG} obtained after adjustment for age, sex and lipid-lowering treatment at baseline.

^bSensitivity analyses excluding individuals on lipid-lowering medication in the lipid analyses and antihypertensive medication in the blood pressure analyses.

DISCUSSION

This population-based study using Mendelian randomization methods found an independent association of β coefficients for FPG with arterial stiffness measured as c-f PWV. Whenever using the individual GRS method, both FPG and T2D were associated with c-f PWV. Multivariable inverse-variance weighted Mendelian randomization was used to correct for potential bias because of pleiotropic effects, which violates a basic assumption of Mendelian randomization. Therefore, the significant results for T2D from individual GRS method in our study is interpreted as an effect of pleiotropy. This is further supported by the T2D41 GRS results – the fact that the T2D GRS lost its significance with c-f PWV after removing seven SNPs also included in the FPG GRS.

Previous studies have found associations between arterial stiffness and diabetes, FPG, triglycerides and HDLc [5–7]. However, the exact mechanisms behind this are unclear and it is not known whether it is an effect of hyperglycemia per se or rather caused by more complex metabolic changes associated with insulin resistance. Arterial stiffness is known to be associated with increasing number of traits of the metabolic syndrome, which supports the role of insulin resistance [27,28]. On the other hand, the increased arterial stiffness in type 1 diabetes points towards a more prominent role of hyperglycemia per se [27]. This is supported by the results from our study where only the genetic disposition of fasting hyperglycemia was associated with arterial stiffness. Even though an increased fasting plasma glucose is one of the T2D criteria, the genetic predisposition of T2D is likely to incorporate several other complex metabolic traits including insulin resistance and abdominal fat accumulation. The SNPs associated with fasting glycemia included in our study, on the other hand, were established in a population without diabetes and should reflect genetic variation of plasma glucose within the normal range with no or weaker tendency of progression to overt T2D. Increased levels of plasma glucose could be harmful to arterial wall through its abilities to facilitate the formation of advanced glycation end-products (AGEs) and enhancement of collagen cross-linking in the arterial wall, decreasing its elastic properties [29,30]. Hyperglycemia has also been shown to induce the angiotensin II production in the arterial wall, a factor relevant for arterial remodeling [31].

Several epidemiological studies have shown that impaired fasting glucose (IFG) is associated with arterial stiffness even in levels below the threshold for a diabetes diagnosis [27,32–34]. This has also been demonstrated in data from the MDC cohort [5]. Such data support our results and indicate that hyperglycemia is harmful to the arterial wall even without the presence of overt diabetes. Our study suggests that macrovascular effects of hyperglycemia could be one pathway, linking plasma glucose to target organ damage and cardiovascular events. On the basis of the results from our study, every treatment, pharmacological or nonpharmacological, that lowers fasting glycemia effectively would have the potential to also reduce arterial stiffening and lower the risk for cardiovascular events and death [3].

The relationship between GRS for T2D and arterial stiffness has previously been investigated in a Mendelian randomization study of 11 385 individuals from Shanghai, China [35]. The results showed a positive relationship between arterial stiffness measured by brachial-ankle PWV and a 34 SNPs GRS for T2D ($OR = 1.24$, $P = 0.008$), thereby showing concurrent results with the individual GRS results from our study.

Strengths of this study include the community-based population and the thoroughly measured outcome, c-f PWV, which is the gold standard for measuring arterial stiffness [36]. As one SNP explains very little of a trait variance, GRSs were used as instrumental variables instead of individual SNPs in order to increase power. However, a GRS may include several SNPs that have pleiotropic effects on other cardiometabolic traits, thus, violating a basic assumption of an instrumental variable in Mendelian randomization [11]. This was addressed by the use of inverse-variance weighted Mendelian randomization, which should reduce this problem [25,26]. Inverse-variance weighted Mendelian randomization method has been applied in previous publications to address the issue of pleiotropy [37,38]. The widespread use of lipid-lowering and blood pressure-lowering drug treatment in our study diluting the effect of genetic variants on the phenotype is problematic. This was addressed both by adjustment and also by sensitivity analyses. The limitations above should, however, not result in any false rejections of the null hypothesis. Therefore, we have focused on the positive rather than negative findings in this study knowing that negative results might be explained by lack of power. Finally, in Mendelian randomization, the genetic variant used as an instrumental variable should be reliably associated to the marker. The SNPs used to construct the GRSs have in meta-analysis been shown to be associated with their respective phenotypes. However, in our cohort, the absence of significant associations between SBP GRS and SBP violates an assumption of Mendelian randomization making SBP GRS an unsuitable instrumental variable [11].

In conclusion, our study shows that SNPs raising fasting plasma glucose but not SNPs associated with T2D were associated with arterial stiffness. This suggests a causal stiffening effect of glycemia on the arterial wall, independently of diabetes status. This is, to our knowledge, the first study investigating the relationship of cardiometabolic SNPs and arterial stiffness using inverse-variance weighted

Mendelian randomization, as a method of accounting for pleiotropy. The absence of significant associations between T2D SNPs and arterial stiffness in our study is, in our opinion, surprising and needs confirmation in future studies.

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Conflicts of interest

There are no conflicts of interest.

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Reviewer's Summary Evaluation

Reviewer 1

Strengths

- To point out that plasma glucose levels, irrespective of the presence of diabetes, may affect arterial stiffness by using the genetic risk scores to support a causal relationship.

- Robust methodological approach; a large number of unique SNPs (183) included in the Mendelian randomization.
- The working hypothesis of lowering fasting glucose to reduce arterial stiffness and, therefore, CV risk.

Weaknesses

- The negative findings on the role of diabetes are likely due to the relatively small cohort and lack of power of the study.