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ORIGINAL ARTICLE

Examining the causal association of fasting glucose with blood pressure in healthy children and adolescents: a Mendelian randomization study employing common genetic variants of fasting glucose

TS Goharian[1], LB Andersen[1,2], PW Franks[3,4], NJ Wareham[5], S Brage[1,5], T Veidebaum[6], U Ekelund[2,5], DA Lawlor[7], RJF Loos[8] and A Grøntved[1]

The aim of the study was to determine whether genetically raised fasting glucose (FG) levels are associated with blood pressure (BP) in healthy children and adolescents. We used 11 common genetic variants of FG discovered in genome-wide association studies (GWAS), including the rs560887 single-nucleotide polymorphism (SNP) located in the *G6PC2* locus found to be robustly associated with FG in children and adolescents, as an instrument to associate FG with resting BP in 1506 children and adolescents from the European Youth Heart Study (EYHS). Rs560887 was associated with increased FG levels corresponding to an increase of 0.08 mmol l^{-1} ($P = 2.4 \times 10^{-8}$). FG was associated with BP, independent of other important determinants of BP in conventional multivariable analysis (systolic BP *z*-score: 0.32 s.d. per increase in mmol l^{-1} (95% confidence interval (CI) 0.20–0.44, $P = 1.9 \times 10^{-7}$), diastolic BP *z*-score: 0.13 s.d. per increase in mmol l^{-1} (95% CI 0.04–0.21, $P = 3.2 \times 10^{-3}$). This association was not supported by the Mendelian randomization approach, neither from instrumenting FG from all 11 variants nor from the rs560887, where non-significant associations of glucose with BP were observed. The results of this study could not support a causal association between FG and BP in healthy children and adolescents; however, it is possible that rs560887 has pleiotropic effects on unknown factors with a BP lowering effect or that these results were due to a lack of statistical power.

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INTRODUCTION

Observational studies in children and adolescents have revealed a positive association between fasting glucose (FG) and blood pressure (BP).[1] Ten percent of adolescents with type 2 diabetes participating in the TODAY trial had hypertension, and the incidence during the average 3.9 years of follow-up was 22%.[2] Nevertheless, treatment with hypoglycemic agents could not lower the incidence of hypertension, and glycemic control was unrelated to the incidence of hypertension in this cohort of adolescents with type 2 diabetes.[3] In adults, higher FG has been found to be associated with subsequent elevated BP in several prospective cohort studies,[4–6] However, some prospective studies do not support this association[7,8] and as impaired FG and raised BP share common environmental and biological risk factors, residual and unknown confounding may explain the observed associations raising the possibility that hyperglycemia is not causally related to high BP.

Some evidence from randomized controlled trials among type 1 diabetics and individuals with impaired glucose metabolism support a causal role for glucose homeostasis in risk of elevated BP. One trial has shown that intensive insulin therapy reduces the long-term risk of hypertension in patients with type 1 diabetes[9] and a second that glucose-lowering therapy in patients with impaired glucose tolerance is effective in reducing the risk of hypertension.[10] Because hypoglycemic agents also target pathways extrinsic to that of glucose homeostasis, findings from these trials could also be explained by other biological factors. Further evidence regarding whether this association is causal would be valuable since it would point to potential interventions for reducing raised FG levels in those at risk of, as well as with, diabetes to prevent hypertension.

Genome-wide association studies (GWAS) have identified several common genomic variants associated with FG.[11] These include a variant (rs560887) located within the third intron of the *G6PC2* locus. *G6PC2* is particularly expressed in pancreatic beta cells. *G6PC2* is thought to hydrolyze glucose-6-phosphate (G6P) to glucose and inorganic phosphate opposing the action of glucokinase (a glucose sensor in beta cells), which facilitates phosphorylation of glucose to G6P in the first step of glycolysis.[12] The risk allele of rs560887 is believed to increase the expression or functional activity of *G6PC2*.[13] As a consequence the flux of glucose into the glycolytic pathway is reduced and this may lead

[1]Research Unit for Exercise Epidemiology, Department of Sport Science and Clinical Biomechanics, Centre of Research in Childhood Health, University of Southern Denmark, Odense M, Denmark; [2]Department of Sports Medicine, Norwegian School of Sport Sciences, Oslo, Norway; [3]Department of Clinical Sciences, Lund University, Malmö, Sweden; [4]Department of Nutrition, Harvard School of Public Health, Boston, MA, USA; [5]Medical Research Council Epidemiology Unit, Institute of Metabolic Science, Cambridge, UK; [6]Centre of Behavioral and Health Sciences, National Institute for Health Development, Tallinn, Estonia; [7]MRC Centre for Causal Analyses in Translational Epidemiology, School of Social and Community Medicine, University of Bristol, Bristol, UK and [8]Department of Preventive Medicine, Charles R. Bronfman Institute of Personalized Medicine, Institute of Child Health and Development, Mount Sinai School of Medicine New York, New York, NY, USA. Correspondence: Dr A Grøntved, Department of Sports Science and Clinical Biomechanics, Centre of Research in Childhood Health, University of Southern Denmark, Campusvej 55, Odense M 5230, Denmark.
E-mail: agroentved@health.sdu.dk
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to a higher glucostatic set-point of the beta cell, possibly leading to a higher FG level as revealed in a recent report from the European Youth Heart Study (EYHS).[14] The effect of this variant has been replicated and found to be robust in children and youth,[14,15] as well as in adults.[13,16,17] Importantly, there is currently no evidence showing that rs560887 has an effect on insulin levels, insulin sensitivity or other biological parameters,[11,13–15] and with no obvious direct role of *G6PC2* in a BP regulating pathway,[11] this variant is an attractive instrument variable for FG. We therefore aimed to examine the association of FG with BP using conventional multivariable-adjusted linear regression and also to examine the causal effect via a Mendelian randomization approach[18] by utilizing the common variant rs560887 in the *G6PC2* gene as well as creating a genetic risk score (GRS) utilizing additional confirmed FG variants from GWAS as instrumental variables (IVs) for FG. We studied this in a population sample of healthy children and adolescent from the EYHS.

MATERIALS AND METHODS

Study population

Data from the EYHS, a multi-country longitudinal population-based study in children, were used in this study. Details of the design and data collection for EYHS have been previously published.[19] DNA, relevant risk factors and outcome data were available from Estonian (city and county of Tartu) and Danish (city of Odense) participants. A proportional two-stage cluster sample of boys and girls aged 9–11 and 14–16 years were selected. In total, 2025 children and adolescents agreed to participate, with a similar proportion participating in each country (76% accepted in Estonia and 75% accepted in Denmark). A total of 1524 had DNA samples available and full data on all variables. Additionally 15 individuals were excluded due to glucose levels $\geqslant 7$ mmol l^{-1} ($n = 3$) or not fasting overnight ($n = 15$) leaving a total of 1506 individuals.

Anthropometry and maturity

Weight, height and waist circumference (WC) were measured while the participants were wearing light clothing, without shoes, using standard techniques.[19] Body mass index (BMI) was calculated as weight (kg)/height^2 (m^2). WC was measured with a metal anthropometric tape midway between the lower rib margin and the iliac crest at the end of gentle expiration. Pubertal status was assessed according to Tanner.[20] Assessment in girls was performed according to breast development and in boys according to pubic hair growth. Maturity stage among the 8- to 10-year olds was almost exclusively stage 1 or 2 in both Danish and Estonian children and almost all adolescents were categorized as Tanner stage 3, 4 or 5. Thus, we collapsed maturity to a 3-point ordinal variable (Tanner 1–2, Tanner 3–4 and Tanner 5).

FG, insulin and lipids

Intravenous blood samples were drawn from an antecubital vein after participants had fasted for at least 8 h. These were separated and stored at −80 °C until analysis. Blood samples were analyzed by one of two Clinical Pathology Accreditation accredited laboratories located in Bristol or Cambridge, England. Glucose, high-density lipoprotein (HDL), total cholesterol (TC) and triglyceride (TG) concentrations were measured by standard methods using Olympus AU600 (Olympus Diagnostica, Hamburg, Germany) random access analyzers. Insulin was analyzed using an enzyme immunoassay (microtiter plate format; Dako Diagnostics, Ely, UK). Between-laboratories correlations were 0.94–0.98 for 30 randomly selected samples analyzed in both Bristol and Cambridge.

Genotyping

Sixteen variants (loci) associated with FG were identified from published meta-analyses.[12,16] Five variants were excluded due to pleiotropic effects, leaving eleven selected variants: rs4607517 (*GCK*), rs340874 (*PROX1*), rs11920090 (*SLC2A2*), rs560887 (G6PC2), rs10885122 (*ADRA2A*), rs2191349 (*DGKB-TMEM195*), rs7944584 (*MADD*), rs7034200 (*GLIS3*), rs13266634 (*SLC30A8*), rs11708067 (*ADCY5*) and rs11071657 (*C2CD4B*). All of the single-nucleotide polymorphisms (SNPs) were genotyped at the Medical Research Centre Epidemiology Unit Research Laboratory in Cambridge, England, using the TaqMan SNP Genotyping Assays (Applied Biosystems, Warrington, UK). The genotyping assay was undertaken on 10 ng of genomic DNA in a 5 µl 384-well TaqMan assay using a PTC-225 Thermal Cycler (MJ Research, Watertown, MA, USA). The ABI PRISM 7900HT Sequence Detection System (Applied Biosystems) was used for end point detection and allele calling. The call rate was >98.9% and concordance of 44 duplicate samples was 100%.

Blood pressure

BP was measured with participants in the up-right sitting position after resting for 5 min. Five measurements were conducted with 2-min intervals between each (Dinamap model XL; Kivex/Critikron, Inc., Tampa, FL, USA). The mean of the last three measurements was used in all analyses. The Dinamap monitor has been validated in children against direct radial artery readings (mean error 0.24 mm Hg systolic blood pressure (SBP) and 1.28 mm Hg diastolic blood pressure (DBP)).[21] BP measurements were converted to age-, height- and sex-specific *z*-scores according to NHBPEP (National High Blood Pressure Education Program) Working Group on Children and Adolescents.[22]

Other covariates

Cardiorespiratory fitness was assessed during a progressive maximal ergometer bicycle test (Ergomedic 839; Monark, Varberg, Sweden) as previously described.[19] Maximal power output (wattmax) per kg body-weight from the test was used to estimate cardiorespiratory fitness. Monthly frequency of soft drinks, fruit and vegetable intake was obtained by self-report. Parental educational level, birth weight and infant breastfeeding status were obtained through a parental questionnaire. Breastfeeding status was, as previously described,[23] expressed in a variable with two categories based on the mother's response: (i) children who were never exclusively breastfed and (ii) those whose mothers indicated that they were never exclusively breastfed. Parental educational level was classified according to International Standard Classification of Education (ISCED) (UNESCO 1997). However, as the details obtained of the description of education were insufficient, the ISCED level 1 and 2 was collapsed to one group, level 3 and 4 was grouped, and level 5, 6 and 7 was grouped as one in the analysis. We used the highest maternal or paternal level of education reported in the analysis.

Statistics

We used multivariable linear regression to first examine the association of FG with BP, adjusting for potential confounding by age, age group, gender, country, insulin, TC, TG, HDL, BMI, WC, intake of soft drinks (Drinks), intake of fruit and vegetable (Fruit/vegetable), cardiorespiratory fitness (Fitness), birth weight, breastfed, maturity and parental education. We also assessed the size and precision of the coefficient of rs560887 (per allele) on BP changes with additional adjustment for FG. We used linear regression to examine the association of individual instruments with FG, adjusting for age, age group, gender and country. We then undertook an IV regression to determine the causal association of FG with BP. The IV analysis was performed using a two-stage least squares regression and the results from the IV regression were compared with that from the standard linear regression using the Durbin form of the Durbin-Wu-Hausman statistic.[24] To evaluate the strength and robustness of rs560887 and the GRS to instrument FG we calculated the first-stage F-statistic from our IV analysis. An *F*-value of >10 has been proposed as a cutoff point for evaluating sufficiently strong instruments in instrument variables regression.[25] We also examined whether rs560887 was associated with any of the observed confounders.

Because we studied two populations with possible different ancestry we explored the possibility for population stratification by conducting the rs560887-FG association separately in each of the two populations and comparing the point estimates before pooling the data in a single analysis. This was done by testing the rs560887-by-country interaction on glucose and evaluating the point estimates for each country. A *P*-value of <0.1 was considered as an indication of interaction. In all analyses, the genetic model used was per allele. All statistical analyses were performed in STATA 11.0 (STATA Corporation, College Station, TX, USA).

Genetic risk scores

The unweighted GRS was calculated by adding the number of risk alleles divided by the number of SNPs, assuming that each allele has an equal effect. The weighted GRS (wGRS) was created by applying weights representing the effect size each allele carries on FG (extracted from the

Barker *et al*.[15] meta-analysis of six studies in healthy children and adolescents). Linear regression was performed, assuming an increase in risk with the accumulation of risk alleles, adjusted for the possible confounders. The IV analysis was performed as described for rs560887. Other than rs340874, none of the SNPs were associated with insulin in this study. The associations of the instruments (rs560887, GRS and wGRS) with FG are shown in Supplementary Table 1.

RESULTS

General characteristics by age group and gender are shown in Table 1. The frequency of the minor allele of rs560887 was 29.9 and 31.0% in Danish and Estonian sample, respectively. Table 2 shows the association of FG and other potential BP determinants with BP. FG was positively associated with BP in the basic model controlling for age, age group, gender and country. The association somewhat attenuated, but remained statistically significant after including all other determinants of BP. FG, insulin, TC, TG, BMI, WC, fitness, intake of fruit and vegetables, birth weight and breastfed were significantly associated with either SBP or DBP in multivariable-adjusted analyses including all potential determinants of BP (Table 2). Supplementary Table 2 details the association of potential confounding factors with rs560887 and Supplementary Table 3 lists associations of potential confounding factors with FG. Intake of drinks and parental education were weakly associated with rs560887, but none of the other confounding factors were associated with rs560887. By contrast, all of the confounding variables, with a few exceptions (HDL, drinks, intake of fruit and vegetables, birth weight and parental education) were associated with glucose.

Association between common genetic glucose variants and FG

We observed a strong association between rs560887 and FG (increase in mmol l^{-1} per allele = 0.08, 95% confidence interval (CI) 0.05–0.11, $P = 2.4 \times 10^{-8}$ adjusting for age, age group, gender and country). The first-stage F-statistics was 31.46. The rs560887 SNP accounted for 2.2% of the variance in FG. When adjusting for all confounders, there was a small increase in significance level ($P = 2.4 \times 10^{-9}$) as well as in the first-stage F-statistics. There was no country-specific association between rs560887 and FG ($P = 0.51$ for interaction). The increase in mmol l^{-1} FG per addition of risk allele was 0.07 (95% CI 0.03–0.11, $P = 6.9 \times 10^{-4}$) for Estonia and 0.09 (95% CI 0.05–0.13, $P = 7.5 \times 10^{-6}$) for Denmark, adjusting for age, age group and gender. The instruments in Estonia were not strong (Supplementary Table 1); thus, sensitivity analyses were performed analyzing each country separately in the IV regression analysis. The associations of individual variants with FG are listed in Supplementary Table 4.

Mendelian randomization approach for the association between glucose and BP

Rs560887 was negatively, and non-significantly associated with SBPz, decreasing SBPz by − 0.04 s.d. (95% CI − 0.11–0.02, $P = 0.22$)

Table 1. Characteristics of the participants by age group and gender, $n = 1506$

	Children (n = *937*)		*Adolescents* (n = *569*)	
	Boys (n = *441*)	*Girls* (n = *496*)	*Boys* (n = *261*)	*Girls* (n = *308*)
Age (years)	9.7 ± 0.5	9.6 ± 0.4	15.5 ± 0.5	15.4 ± 0.5
Height (cm)	139.0 ± 6.5	138.9 ± 6.8	174.3 ± 7.1	164.7 ± 5.9
Weight (kg)	33.2 ± 6.0	33.3 ± 6.9	63.0 ± 9.8	55.7 ± 8.1
BMI (kg m^{-2})	17.1 ± 2.2	17.2 ± 2.6	20.6 ± 2.5	20.5 ± 2.6
WC (cm)	59.4 ± 5.4	58.6 ± 6.8	71.5 ± 5.7	66.6 ± 5.5
Maturity[a]—No (%)				
1	441 (100.00)	483 (97.4)	10 (3.8)	2 (0.7)
2	—	13 (2.6)	107 (41.0)	165 (53.6)
3	—	—	144 (55.2)	141 (45.8)
SBP (mm Hg)	102.9 ± 8.8	101.1 ± 8.7	116.5 ± 11.1	108.1 ± 9.1
SBPz	0.05 ± 0.8	− 0.09 ± 0.8	0.02 ± 1.0	− 0.58 ± 0.9
DBP (mm Hg)	60.2 ± 7.0	60.2 ± 6.4	63.3 ± 6.7	64.1 ± 6.5
DBPz	− 0.09 ± 0.6	− 0.05 ± 0.6	− 0.20 ± 0.6	− 0.20 ± 0.6
Glucose (mmol l^{-1})	5.1 ± 0.3	5.0 ± 0.4	5.2 ± 0.4	5.0 ± 0.4
Insulin (pmol l^{-1})	39.8 ± 22.2	46.5 ± 24.7	70.9 ± 35.3	78.6 ± 37.4
TC (mmol l^{-1})	4.5 ± 0.7	4.6 ± 0.8	4.0 ± 0.7	4.4 ± 0.8
HDL (mmol l^{-1})	1.6 ± 0.3	1.5 ± 0.3	1.3 ± 0.3	1.4 ± 0.3
TG (mmol l^{-1})	0.7 ± 0.3	0.8 ± 0.3	0.8 ± 0.3	0.9 ± 0.4
Fruit/vegetable (servings/month)	35.6 ± 16.5	38.5 ± 15.2	30.5 ± 16.1	34.8 ± 16.4
Drink (servings/month)	9.8 ± 9.3	7.4 ± 7.5	12.6 ± 9.6	8.9 ± 8.4
Fitness (watts/kg)	3.2 ± 0.5	2.8 ± 0.5	3.6 ± 0.5	2.7 ± 0.5
Birth weight (g)	3500 ± 620	3404 ± 612	3519 ± 599	3320 ± 533
Breastfed—No (%)	374 (84.8)	430 (86.7)	218 (83.5)	240 (77.9)
Parental education[b]—No (%)				
1	29 (6.6)	24 (4.8)	15 (5.8)	27 (8.8)
2	112 (25.4)	132 (26.6)	68 (26.0)	74 (24.0)
3	300 (68.0)	340 (68.6)	178 (68.2)	207 (67.2)
GRS	16.7 ± 2.2	16.7 ± 2.2	16.8 ± 2.1	16.8 ± 2.1
wGRS	28.2 ± 4.2	28.3 ± 4.2	28.0 ± 4.6	28.3 ± 4.3

Abbreviations: BMI, body mass index; DBP, diastolic blood pressure; GRS, unweighted risk score; HDL, high-density lipoprotein cholesterol; SBP, systolic blood pressure; TC, total cholesterol; TG, triglycerides; WC, waist circumference; wGRS, weighted risk score. Data are means ± s.d. or %. [a]Maturity 1 = Tanner stage 1–2, Maturity 2 = Tanner stage 3–4 and Maturity 3 = Tanner stage 5. [b]According to International Standard Classification of Education (ISCED) (UNESCO 1997). Parental education 1 = ISCED level 1–2, Parental education 2 = ISCED level 3–4 and Parental education 3 = ISCED level 5–7.

Table 2. Associations of fasting glucose, and potential confounders, with blood pressure, $n = 1506$

	Difference in SBPz per unit of category of exposure			*Difference in DBPz per unit of category of exposure*		
	Effect size	*95% CI*	P-*value*	*Effect size*	*95% CI*	P-*value*
Adjusted for country, age, age group and gender						
Glucose (mmol l^{-1})	0.39	0.27; 0.51	$2.\times10^{-10}$	0.18	0.10; 0.26	1.1×10^{-5}
Insulin (pmol l^{-1})	0.01	3.9×10^{-3}; 0.01	1.4×10^{-12}	2.6×10^{-3}	1.6×10^{-3}; 3.6×10^{-3}	7.3×10^{-7}
TC (mmol l^{-1})	0.11	0.05; 0.17	1.4×10^{-4}	0.09	0.05; 0.13	3.5×10^{-6}
TG (mmol l^{-1})	0.39	0.26; 0.51	4.6×10^{-9}	0.28	0.20; 0.37	1.7×10^{-10}
HDL (mmol l^{-1})	−0.09	−0.23; 0.05	0.20	2.6×10^{-3}	−0.09; 0.10	0.96
BMI (kg m^{-2})	0.08	0.06; 0.09	5.7×10^{-17}	0.01	-4.2×10^{-3}; 0.02	0.20
WC (cm)	0.02	0.01; 0.03	1.5×10^{-8}	1.8×10^{-3}	−0.01; 3.2×10^{-3}	0.48
Drinks (servings/month)	2.6×10^{-3}	2.5×10^{-3}; 0.01	0.31	2.1×10^{-3}	1.3×10^{-3}; 0.01	0.23
Fruit/vegetable (servings/month)[a]	2.3×10^{-3}	−0.01; 4.5×10^{-4}	0.10	1.1×10^{-3}	3.0×10^{-3}; 7.5×10^{-4}	0.24
Fitness (watts/kg)	−0.10	−0.18; −0.01	0.03	−0.05	−0.11; 0.01	0.09
Birth weight (g)	-1.0×10^{-4}	-1.8×10^{-4}; -3.0×10^{-5}	5.9×10^{-3}	-3.0×10^{-6}	-5.3×10^{-5}; 4.7×10^{-5}	0.91
Breastfed	−0.18	−0.30; −0.06	0.003	−0.07	−0.15; 0.01	0.09
Maturity[b]						
1	reference	—	—	reference	—	—
2	0.13	−0.22; 0.47	0.47	−0.04	−0.27; 0.20	0.75
3	0.50	0.14; 0.86	0.01	0.17	−0.07; 0.42	0.17
Parental education[c]						
1	reference	—	—	reference	—	—
2	−0.04	−0.23; 0.16	0.69	−0.04	−0.17; 0.09	0.53
3	−0.08	−0.26; 0.11	0.40	−0.09	−0.22; 0.04	0.16
Multivariable adjusted[a]						
Glucose (mmol l^{-1})	0.32	0.20; 0.44	1.9×10^{-7}	0.13	0.04; 0.21	3.2×10^{-3}
Insulin (pmol l^{-1})	2.0×10^{-3}	3.9×10^{-4}; 3.6×10^{-3}	0.02	1.3×10^{-3}	1.9×10^{-4}; 2.5×10^{-3}	0.02
TC (mmol l^{-1})	0.07	0.01; 0.14	0.03	0.06	0.01; 0.11	0.01
TG (mmol l^{-1})	0.12	−0.03; 0.26	0.11	0.18	0.08; 0.29	4.0×10^{-4}
HDL (mmol l^{-1})	−0.05	−0.22; 0.11	0.53	2.4×10^{-4}	−0.12; 0.11	1.0
BMI (kg m^{-2})	0.11	0.07; 0.14	1.8×10^{-10}	0.02	-3.0×10^{-3}; 0.04	0.09
WC (cm)	−0.01	−0.03; 2.2×10^{-4}	4.6×10^{-2}	−0.01	−0.02; −0.01	1.7×10^{-3}
Drinks (servings/month)	1.3×10^{-3}	3.6×10^{-3}; 0.01	0.61	9.2×10^{-4}	-2.5×10^{-3}; 4.3×10^{-3}	0.60
Fruit/vegetable (servings/month)[a]	2.8×10^{-3}	−0.01; 9.5×10^{-5}	0.04	8.1×10^{-4}	-2.7×10^{-3}; 1.0×10^{-3}	0.39
Fitness (watts/kg)	0.16	0.07; 0.26	1.0×10^{-3}	−0.02	−0.08; 0.05	0.65
Birth weight (g)	1.2×10^{-4}	1.9×10^{-4}; 5.0×10^{-5}	9.0×10^{-4}	2.0×10^{-5}	-3.0×10^{-5}; 7.0×10^{-5}	0.43
Breastfed	−0.15	−0.27; −0.04	7.9×10^{-3}	−0.05	−0.13; 0.03	0.21
Maturity[b]						
1	reference	—	—	reference	—	—
2	−0.06	−0.39; 0.27	0.72	−0.04	−0.28; 0.19	0.71
3	0.20	−0.15; 0.55	0.26	0.15	−0.10; 0.39	0.24
Parental education[c]						
1	reference	—	—	reference	—	—
2	−0.04	−0.23; 0.14	0.66	−0.03	−0.16; 0.10	0.62
3	−0.02	−0.20; 0.15	0.79	−0.06	−0.18; 0.07	0.38

Abbreviations: BMI, body mass index; DBP, diastolic blood pressure; HDL, high-density lipoprotein cholesterol; PE, parental education; SBP, systolic blood pressure; TC, total cholesterol; TG, triglyceride; WC, waist circumference. [a]Adjusted for country, age, age group, gender, maturity, parental education, birth weight, breastfed, BMI, waist circumference, triglycerides, total cholesterol, HDL, insulin, fitness, intake of fruit and vegetables and intake of soft drinks. [b]Maturity 1 = Tanner stage 1–2, Maturity 2 = Tanner stage 3–4 and Maturity 3 = Tanner stage 5. [c]According to International Standard Classification of Education (ISCED) (UNESCO 1997). Parental education 1 = ISCED level 1–2, Parental education 2 = ISCED level 3–4 and Parental education 3 = ISCED level 5–7.

per allele, adjusted for age, age group, country and gender. A similar association was found with the GRS, decreasing SBPz with −0.02 s.d. per increase in risk alleles for GRS (95% CI −0.04–0.004, $P = 0.11$) and −0.01 s.d. for wGRS (95% CI −0.03–-0.01, $P = 0.21$). After adjusting for glucose, the effect on BP augmented and the *P*-value became significant, decreasing SBPz with −0.07 s.d. (per allele) (95% CI −0.14 to −0.01, $P = 0.03$) for rs5608887, −0.03 s.d. (95% CI −0.05 to −0.01, $P = 0.01$) for GRS and −0.02 s.d. (95% CI −0.04 to 0.004, $P = 0.02$) for wGRS.

Table 3 presents the results from the IV analyses and compares these with the results from the conventional multivariable regression analyses. Despite the positive association from the conventional FG-BP multivariable regression, the IV analysis using rs560887, GRS and wGRS showed a negative non-significant association with SBPz. The estimates from the IV approach on SBPz were significantly different from the conventional multivariable analysis (*P*-value for difference < 0.05). Similar results were found when analyzing each country separately (results not shown).

Power calculation

We carried out a *post hoc* power calculation,[26] which suggest that with $n = 1506$ an explained variance for the regression of the

Table 3. Instrumental variable analyses of the association of fasting glucose with blood pressure, using rs560887 and genetic risk scores, $n = 1506$.

	Difference in SBPz per increase in mmol l^{-1} FG			P-*value for difference with conventional approach*[a]	*Difference in DBPz per increase in mmol l^{-1} FG*			P-*value for difference with conventional approach*[a]
	Effect size	*95% CI*	P-*value*		*Effect size*	*95% CI*	P-*value*	
IV analysis, rs560887[b]	−0.54	−1.41; 0.33	0.22	0.03	4.2×10^{-3}	−0.59; 0.58	0.99	0.51
IV analysis, rs560887[c]	−0.53	−1.36; 0.30	0.21	0.03	−0.04	−0.62; 0.54	0.90	0.48
IV analysis, GRS[b]	−0.61	−1.37; 0.14	0.11	0.01	−0.04	−0.56; 0.47	0.87	0.37
IV analysis, GRS[c]	−0.55	−1.28; 0.17	0.14	0.01	−0.06	−0.56; 0.459	0.82	0.38
IV analysis, wGRS[b]	−0.47	−1.20; 0.26	0.21	0.02	−0.10	−0.59; 0.40	0.71	0.25
IV analysis, wGRS[c]	−0.46	−1.16; 0.25	0.20	0.02	−0.17	−0.66; 0.32	0.49	0.17
MV analysis[b]	0.39	0.27; 0.51	< 0.0001		0.18	0.10; 0.26	< 0.0001	
MV analysis[c]	0.32	0.20; 0.44	< 0.0001		0.13	0.04; 0.21	0.003	

Abbreviations: CI, confidential interval; DBP, diastolic blood pressure; FG, fasting glucose; GRS, unweighted genetic risk score; HDL, high-density lipoprotein; IV, instrumental variable; MV, multivariable; SBP, systolic blood pressure; wGRS, weighted genetic risk score. [a]*P*-value for difference in estimates between results from the IV approach and the conventional approach (MV analysis), using Durbin-Wu Hausman. [b]Adjusted for country, age, age group and gender. [c]Adjusted for country, age, age group, gender, maturity, parental education, birth weight, breastfed, body mass index, waist circumference, triglycerides, total cholesterol, HDL, insulin, fitness, intake of fruit and vegetables and intake of soft drinks.

exposure on the genetic variant of 2.2% (rs560887), a crude association of glucose with SBP of 0.42 s.d. per mmol l^{-1}, and an estimated causal effect of glucose on SBP of 0.32 s.d. per mmol l^{-1}, yield a power of 12% with alpha = 0.05.

DISCUSSION

We determined the association of FG and BP with the help of the rs560887 common allele in the *G6PC2* locus and a GRS consisting of 11 SNPs, previously shown to be associated with FG in adults,[11] children and adolescents.[14,15] In a population sample of European children and adolescents, FG in the non-diabetic range was positively associated with SBPz and DBPz in multivariable analyses adjusted for major lifestyle, socio-demographic and biological determinants of FG, suggesting that the association was not a consequence of known confounders. In contrast, genetically elevated FG either instrumented from rs560887 or all FG SNPs did not show an association with BP. In fact, we observed evidence that associations differed between conventional analyses and the Mendelian randomization approach. Collectively, our results from the Mendelian randomization approach could not confirm that FG is causally related to BP among healthy children and adolescent of European descent.

Comparison with other studies

Our findings from the conventional analysis are in line with previous observational studies in children and adolescents supporting a positive association between FG and BP, and with studies comparing BP levels between diabetic and non-diabetic children and adolescents.[1,27,28] Furthermore, the majority of previous observational studies among healthy adults suggest a positive association between FG and BP levels.[4–6] We are not aware of any studies that have reported associations of genetically elevated FG with BP levels or hypertension; however, other Mendelian randomization analyses describing associations of glucose levels and related outcomes have been reported. A recent study among Danish men and women found that genetically elevated non-FG levels were associated with an increased risk of coronary heart disease independent of hypertension and other biological risk factors for cardiovascular disease.[29] A study among US men and women found that a GRS based on five GWAS FG variants was associated with intima media thickness in the same direction as influencing FG,[17] however, it was not reported whether BP explained this association. Although these studies suggest a causal effect of elevated glucose levels on development of atherosclerosis and coronary heart disease, further Mendelian randomization studies with BP or hypertension are warranted to further refute or support the notion that elevated glucose levels are causally related to raised BP levels.

Limitations with Mendelian randomization

First, if the genetic determinants of FG have pleiotropic effects (exert effects on other factors that are related to BP regulation), our results could be biased. Thus, an alternative explanation for our observations could be that the rs560887 common allele increases FG levels as well as decreases BP. Because we did not find associations of rs560887 or the GRS with any other major determinants of FG, we are fairly confident that this is not the case. Second, another limitation of Mendelian randomization approach is the use of weak instruments. The first-stage F-statistics for rs560887, GRS and wGRS were high, confirming a strong instrument. Third, population stratification could lead to spurious results. Sensitivity analyses confirmed that genetic effects of SNPs on FG were fairly similar between countries and because both populations are of European descent and frequency of the minor allele was similar in both populations, the existence of population stratification is unlikely.[18] Fourth, the IV analysis assumes no interaction and linearity in each stage, and even though our investigations suggested linearity these assumption could still be violated. Finally, our study could be insufficiently powered to determine valid results from the Mendelian randomization approach. This is indicated by the *post hoc* power calculation, revealing only 12% power. Thus, it is probable that are our study is underpowered. These limitations may be plausible causes of the discordant associations between conventional analyses and the Mendelian randomization approach; however, the conventional relation between FG and BP may also be explained by residual/unknown confounding and reverse causation.

Strengths of the study

One of the strengths of this study is the use of a common variant (rs560887) robustly associated with FG. Further, no evidence of pleiotropy from our own investigations or other studies has been found on this SNP. Another strength of this study is that the study population consists of individuals in the early stages of life

(healthy children and adolescents). Studies with an adult population may have a higher prevalence of preclinical diseases (due to accumulation of environmental and behavioral influences), potentially interfering with a possible causal association. However, if the cumulative effect of common genetic glucose variants on FG adds up with time, there is a chance that we could detect effects of larger magnitude in an adult population, as suggested by Renstrom *et al.*

CONCLUSION

Although conventional multivariable-adjusted analyses showed that FG was positively associated with BP among healthy children and adolescents, this association could not be confirmed by the Mendelian randomization approach using either the common variant rs560887 in the *G6PC2* locus or several FG SNPs jointly as instruments. Because, our study was moderate in size we cannot exclude that our finding was due to statistical power reasons and additional studies among populations of children, youth and adults are needed to further exclude this possibility.

What is known about topic

- Higher fasting glucose (FG) has been found to be associated with elevated blood pressure (BP).
- Genome-wide association studies (GWAS) have identified rs560887 as a common genomic variant associated with FG, yielding the opportunity to use this variant as an instrument variable.

What this study adds

- Conventional multivariable-adjusted analyses confirmed a positive association between FG and BP but a negative, non-significant association utilizing the Mendelian randomization approach.
- The effect of rs560887 was confirmed robust in this study population consisting of healthy children and adolescents of European descendants, with FG levels in the non-diabetic range.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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