Relationship between Glucose Homeostasis and Obesity in Early Life – a Study of Italian Children and Adolescents

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Abstract

Objectives

Epidemic obesity is the most important risk factor for prediabetes and type 2 diabetes (T2D) in youth as it is in adults. Obesity shares pathophysiological mechanisms with T2D and is likely to share part of the genetic background. We aimed to test if weighted genetic risk scores (GRSs) for T2D, fasting glucose (FG) and fasting insulin (FI) predict glycaemic traits and if there is a causal relationship between obesity and impaired glucose metabolism in children and adolescents.

Design and patients

Genotyping of 42 SNPs established by genome-wide association studies for T2D, FG and FI was performed in 1,660 Italian youths aged between 2 and 19 years. We defined GRS for T2D, FG and FI and tested their effects on glycaemic traits, including FG, FI, indices of insulin resistance/beta cell function, and body mass index (BMI). We evaluated causal relationships between obesity and FG/FI using one-sample Mendelian Randomization analyses in both directions.

Results

GRS-FG associated with FG (beta=0.075 mmol/l, SE=0.011, P=1.58×10^-11) and beta cell function (beta=-0.041, SE=0.0090 P=5.13×10^-6). GRS-T2D also demonstrated an association with beta cell function (beta=-0.020, SE=0.021 P=0.030). We detected a causal effect of increased BMI on levels of FI in Italian youths (beta=0.31 ln(pmol/l), 95%CI [0.078, 0.54], P=0.0085), while there was no effect of FG/FI levels on BMI.
Conclusion

Our results demonstrate that the glycaemic and T2D risk genetic variants contribute to higher FG and FI levels and decreased beta cell function in children and adolescents. The causal effects of adiposity on increased insulin resistance are detectable from childhood age.

Introduction

Childhood obesity is growing around the globe. In some developed countries, the disease incidence plateaued, nonetheless the rate of severe obesity had increased worldwide (1). In Italy, the prevalence of overweight and 3rd grade obese children has decreased from 35.2% in 2008 to 30.6% in 2016, while the rate of severely obese has reached 2.1% (2). This caused the onset of health conditions previously considered exclusively adult diseases, such as prediabetes (impaired fasting glucose, IFG and glucose tolerance, IGT) and type 2 diabetes (T2D), at an earlier age.

Obesity, especially in children, is a major risk factor of T2D, and urges its study for diabetes prevention (3). Strong evidence suggests that pathogenic mechanisms are shared between obesity, prediabetes and T2D as well as causal effect of body adiposity on hyperinsulinaemia and T2D in adults (4). The rates of their occurrence differ among ethnicities, consistent with differences in genetic susceptibility to T2D, currently less characterized in youths than in adults. In Italy, 3% of adolescents with moderate and severe obesity present IFG and 5% IGT, while T2D is diagnosed in less than 0.5% of adolescents (5). An increased amount of adipose tissue leads to more severe insulin resistance (IR) via different mechanisms (i.e. lipotoxicity, release of some adipokines etc.), thus promoting the development of T2D (6). The early-life adiposity levels correlate with adulthood measures and might therefore represent a longitudinal causal risk factor for adult metabolic health deterioration, which
requires further insight from studies dissecting health of young individuals (7).

To date, over 400 genetic variants have been implicated in the development of T2D and more than 500 genomic loci were uniquely associated with body mass index (BMI) (8-11). In general, these loci have small effect sizes, explaining approximately 6% of trait variance and disease susceptibility (12) when combined. Studies in children (13) and young adults (14-20) have demonstrated that genetic risk scores (GRSs) show stronger predictive ability in younger individuals than in older ones (21-25). Several obesity genes harbour loci that are associated with T2D, but affect T2D susceptibility largely through their effect on BMI via increased IR (e.g. FTO and MC4R gene variants) (26). However, their effects on glucose homeostasis might be overestimated in the context of obesity (27).

Dissection of the genetic effects on the quantitative endophenotypes of T2D, including fasting glucose (FG), and insulin (FI), indices of beta cell function (HOMA-B) and IR (HOMA-IR) in individuals without diabetes, helps uncovering the disease pathophysiology (28). The Meta-Analyses of Glucose and Insulin-related traits Consortium (MAGIC) identified sixteen loci associated with FG/HOMA-B, and two loci associated with FI/HOMA-IR (29). Additional evidence was provided for 24 FG loci (12).

In this paper, we calculated GRS to combine the effects of multiple genetic variants to increase the power of the study. The choice of loci was based on reported associations of genetic variants with FG, FI and T2D (30).

The selection of SNPs for this study was based on prioritisation of the variants with the largest effect size of the phenotype of interest. These variants, including TCF7L2, FTO, MTNR1B, and G6PC2, were most frequently identified in the earliest GWAS studies (Supplementary Table 1) and confirmed by multiple replications (28, 31). This approach allowed us to detect the effect in individuals of young age, when genetic
effects are usually smaller compared to adulthood. We hypothesized that the GRSs for FG, FI and T2D are associated with glycaemic traits, such as IR and beta cell function measured by HOMA-IR/HOMA-B, and adiposity (BMI) as a quantitative measure of obesity in children and adolescents. The aim of our study was to analyse 42 loci with the largest effects on T2D and glycaemic traits in a cohort of children enrolled at the Bambino Gesù Children’s Hospital in Rome, Italy, and to define the effects of these variants on glycaemic traits and indices of IR, beta cell function, and BMI as well as to evaluate the causal relationships between these traits using genetic variants as instruments in a one-sample Mendelian Randomization (MR) framework.

Results

Our study included 1,660 young European-descent individuals from Italy with a mean BMI of 20.72 kg/m² (range 10.42 to 44.95 kg/m²) and a mean age of 9.09 years (range 2.02 to 18.93 years) (Table 1). Most (1,638; 98.67%) of the participants had FG level below 5.6 mmol/l. Twenty (1.20%) participants had FG values between 5.6 and 6.1 mmol/l and two (0.12%) had values between 6.1 and 7.0 mmol/l. Most of them fell within the BMI range between -2 and 2 of gender- and age-specific standard deviation scores (SDS) of BMI units (Material and Methods) with the exception of 205 individuals (12.35%) who were obese (BMI values ≥ 2SD). These individuals with obesity were older and had higher FG, FI, HOMA-B, and HOMA-IR values when compared to non-obese individuals.

We performed the association analyses with FG, FI, HOMA-B, HOMA-IR, and BMI SDS for the 42 variants, assuming an additive genetic model implemented in linear regression and detected nominally significant associations (P<0.05) at eight FG, eight T2D, and two FI loci with
the phenotypes tested (Material and Methods, Supplementary Table 1). For FG, we observed the most significant associations with rs560887 near G6PC2 (glucose-6-phosphatase 2) \((P=7.35\times10^{-6})\), rs4607517 near GCK (glucokinase) \((P=3.24\times10^{-5})\), and rs10830963 at MTNR1B (melatonin receptor 1B) \((P=4.37\times10^{-5})\), of which the signals at G6PC2 and MTNR1B were also associated with HOMA-B \((P=1.13\times10^{-2}\) and \(P=1.32\times10^{-4}\), respectively). Additionally, signals within MADD (MAP Kinase Activating Death Domain) and CHCHD9/TLE4 (Coiled-Coil-Helix-Coiled-Coil-Helix Domain Containing 9/transducin-like enhancer protein 4) were nominally associated with FG. For HOMA-B, we further observed an association with rs10440833 at CDKAL1 (CDK5 regulatory subunit-associated protein 1-like 1, \(P=9.72\times10^{-3}\)). For FI, the variant rs340874 at PROX1 (Prospero Homeobox 1) showed a nominal association \((P=2.37\times10^{-2})\). Furthermore, variation at PPARG (Peroxisome Proliferator Activated Receptor Gamma) was associated with FI \((P=4.49\times10^{-2})\), HOMA-B \((P=2.15\times10^{-3})\) and HOMA-IR \((P=4.01\times10^{-2})\). HOMA-IR was further associated with rs12970134 at MC4R (melanocortin 4 receptor) \((P=3.25\times10^{-2})\). Variation at MC4R was also associated with age- and gender-standardised BMI \((P=8.45\times10^{-3})\), as were rs9939609 at FTO (fat mass and obesity-associated) and rs11558471 at SLC30A8 (Solute Carrier Family 30 Member 8), \((P=1.81\times10^{-2}\) and \(P=2.91\times10^{-2}\), respectively).

The number of FG risk alleles increased, concentrations of FG increased whereas values of HOMA-B decreased (Figure 1). However, contrary to our study hypothesis, the BMI SDS measurement remained relatively invariable compared to the number of FG risk alleles. For the number of FI
risk alleles, no significant association was observed with either FI, HOMA-IR, or BMI SDS (Figure 2). Similar results were obtained for the relationship between T2D risk alleles and FG, FI, HOMA-B, HOMA-IR, and BMI SDS (Figure 3).

When grouping the phenotype distributions according to percentiles, we observed that the individuals in the highest (>95%) percentile group of FG had a slightly increased number of FG risk alleles (mean_{group1}=22.86, mean_{group2}=23.45, mean_{group3}=23.84; P=0.026 in the linear regression of the number of FG risk alleles on the percentile group), whereas the opposite was true for HOMA-B (mean_{group1}=23.84, mean_{group2}=23.46, mean_{group3}=22.61; P=0.0050). No such effect was detected for the BMI SDS percentile groups (mean_{group1}=23.67, mean_{group2}=23.43, mean_{group3}=23.16; P=0.24) (Figure 4).

A higher number of FG risk alleles was associated with elevated FG (beta=0.075, 95%CI [0.053;0.097] mmol/l per unit increase in the weighted GRS) and lower HOMA-B values (beta=-0.041, 95%CI [-0.059; -0.024] ln(HOMA-B) units per unit increase in the weighted GRS) after adjustment for age, sex, and BMI SDS (Material and Methods, Table 2). The weighted T2D GRS was associated with lower HOMA-B values (-0.020 [-0.038; -0.0019]) after adjusting for age, sex, BMI SDS (Table 2). The unweighted GRS provided mostly similar but weaker associations than the weighted GRS (Table 2), except for the unweighted FG GRS which showed a trend towards a negative association with FI levels (-0.024 [-0.050, 0.0029] ln(pmol/l), P=0.082) and this was further strengthened (-0.028 [-0.055, -0.0016] ln(pmol/l), P=0.038) when adding adjustment for family history of T1D and T2D. It is worth noticing that this additional adjustment had in general no noticeable effect on the other estimates (Material and Methods, Supplementary Table 2). We did not observe any evidence for association between the FG and FI GRSs and BMI SDS or obesity status, including adjustments for sex, age, BMI SDS and family history of T1D and T2D (Table 3).
We evaluated the associations between FG/FI and BMI for causality in a bi-directional one-sample MR framework (Material and Methods). We identified a positive causal effect of BMI on FI ($P=0.0085$) (Table 4). The IV estimator indicated a causal effect of 0.31 ln(pmol/l) higher FI (95%CI [0.078;0.54]) per unit increase in BMI SDS. We did not observe a causal effect in the other direction. Contrary to the observed epidemiological associations, we did not find evidence for a causal effect of BMI on the levels of FG and vice versa. However, this could be due to low power as the epidemiological effect estimates between BMI and FG in either direction are lower than those between BMI and FI (Table 4). Additionally, the low F-statistic of the FI IV (Table 4) suggests weak instrument bias and low power of the MR analysis testing the effect of FI levels on BMI.

**Discussion**

Our results confirm the ability of a GRS combining 20 independent genetic variants, associated with FG in previous GWAS (12, 28, 32, 33), to predict values of fasting glucose and beta cell activity in children and adolescents already. Using a one-sample MR approach, we discovered that a causal effect of adiposity via BMI on FI levels is detectable as early as in childhood/teenage years.

**Effects of adiposity on altered glucose metabolism**

The present investigation expands on earlier findings in adults on FTO/MC4R-mediated adiposity’s effect on increased fasting insulin levels (34, 35) to its earlier age manifestation in children and adolescents. Despite much larger sample sizes in earlier adult studies, the causal effect of adiposity on FI in our study is comparable to that in adults (35). While we were able to observe a causal effect in the combined cohort, we were...
underpowered to perform sex-stratified analyses and to validate the larger causal effect of BMI on FI reported in men compared to women (4). This study did not find evidence for a causal effect of adiposity on FG, possibly due to lack of statistical power. The lack of causal effect of BMI on FG is in contrast to the findings of Dale et al. and Xu et al. who report a causal effect of BMI on glucose levels with markedly lower causal effect size than that between BMI and insulin (36, 37). Even though our causal analyses from BMI to FI and FG used only two variants, namely those within FTO and MC4R, to date, these are the most strongly associated with BMI and have been successfully used as instrumental variables to estimate causal effects previously (4, 38). The F-statistic of the BMI instrument (Table 4) demonstrates that these two variants make a strong enough IV in this study. Our findings support a crucial role of adiposity in the development of IR in young individuals. During puberty, IR is changing drastically, when insulin sensitivity undergoes a decline of around 25–50% during puberty and improves when puberty ends (39, 40). Visceral and subcutaneous adipose tissue secrete free fatty acids and proinflammatory cytokines into blood which contribute to IR (41). Changes related to increased adiposity affect the complex interplay between pathophysiological processes already in early age, which might increase the risk of early onset T2D and comorbidities.

**Genetic variants associated with adiposity**

Our findings are in line with previous studies that linked genetic variants near MC4R and FTO genes with adiposity traits and T2D (42). MC4R rs12970134 is associated with increased risk of T2D and higher BMI in both European and trans-ethnic studies (8, 26). The Nord-Trondelag Health (HUNT) study assessing gene-environment interactions of FTO and MC4R on obesity in people with extreme phenotypes observed age- and gender-dependent associations of rs9939609 (FTO) and rs17782313 (MC4R) loci with BMI (43). Notably, the effect sizes of FTO tended to
be the highest in the youngest age group for both genders; for MC4R, the highest effect on BMI was observed in the youngest age group, dipping in the middle age and increasing again after the age of sixty, while in men it peaked at 40-60 years and became negligible later in life (43). The FTO locus is not only strongly associated with T2D (42) and higher BMI (44), but also with higher FI and HOMA-IR (29). These observations are in agreement with BMI playing a role in the FTO association with T2D via IR.

**Effects on altered glucose metabolism in adult and pediatric populations**

In our analysis, the GRS for FG comprising 20 DNA variants explained 2.76% of the variability in FG (beta=0.075mmol/l, P=1.58×10⁻¹¹) and 0.34% of the variability in β-cell function (beta=0.042, P=5.13×10⁻⁶). A number of studies (13-20, 45) evaluated the performance of glucose homeostasis GRSs as a useful tool to estimate the effects of multiple risk alleles predicting prevalent or incident cases of T2D. Similarly, in cross-sectional studies of normal-weight and overweight/obese children, T2D and FI GRSs comprising 62 (23) and 53 (9) SNPs were associated with different glycaemic traits, particularly with FG and estimates of beta cell function.

**Genetic variants associated with fasting glucose and β-cell function**

We confirmed previously established associations of some of the T2D susceptibility variants with glucose metabolism traits (Supplementary table 1). Specifically, among 20 loci previously implicated in FG variability, G6PC2, GCK and MTNR1B variants showed effects on FG in Italian children consistent with those found in European children in MAGIC (28, 29) and in adults (46). According to our results and previous studies, two of these genes, G6PC2 and MTNR1B, have been found to be associated with β-cell function in adults (47, 48). Increased MTNR1B expression in individuals at risk of T2D suggests a direct inhibitory effect on beta cells (47). Our results demonstrate a significant association
between *MADD* and *PPARG* loci and HOMA-B, consistent with previous reports (49, 50). Our study provides evidence that *CDKAL1* variants confer risk of T2D through reduced insulin secretion, which is also in line with the findings of the genome-wide association study in European and Hong Kong populations (51).

**Study limitations**

We acknowledge the limitations of our study of which the limited sample size and the wide age range are the most evident. Other limitations include the cross-sectional design; the weights taken from older populations and effect sizes for risk variants can vary between different age groups and might not provide as good a predictive ability; the limited number of variants investigated; the proxy estimation of fasting IR and β-cell function; the lack of information on pubertal status and the enrolment of individuals with exclusively European descent. In an attempt to mitigate the limitation of wide age range, we used gender and age-specific standardized obesity indices (BMI SDS). Future investigations must consider the changing physiology and hormonal levels during pubertal transition; they should enroll individuals of non-European descent to better reflect the evolving multi-ethnic nature of populations in Italy and worldwide; and the FG GRS should be validated in longitudinal studies. Additionally, the GRSs in our study are constructed based on a relatively small number of variants from pioneer GWAS studies. Therefore, as more variants are characterised, improved GRSs should be constructed.

**Conclusion**

We report that T2D risk genetic variants contribute to higher FG levels and beta cell function in Italian children and adolescents. We provide novel evidence for a causal effect of childhood adiposity on higher FI levels validating previously published results in adults. Further larger
studies in children are mandatory to expand present knowledge of the genetic overlap between childhood/adolescent age obesity and risk of T2D.

Material and methods

Study sample

The study sample comprised individuals referred to the Bambino Gesù Children’s Hospital in Rome, Italy hospital between July 2012 and July 2013 by general practitioners from the Metropolitan area of Rome (Italy) to participate in “The Bambino Gesù Study: Profiling the genetic risk of complex diseases in the Italian population”. The primary aim of the study was to dissect the genetic architecture of glucose homeostasis in the Italian children and adolescents. The study was approved by the Ethics Committee of the Bambino Gesù Hospital, and written informed consent was obtained from the child’s parents or legal guardians in accordance with the Helsinki declaration (52).

In total, 1,806 participants were enrolled in the study. We excluded participants below 2 or above 19 years of age (N=10), non-Europeans (N=135), and one individual with an FG value ≥7 mmol/l. The final study sample included 1,660 (889 male) participants of European descent aged between 2 and 19 years (Supplementary Figure 1). None of the participants were following a weight loss diet or an intensive exercise program, and, at the time of enrollment, all study participants were healthy. Information on family history of diabetes in the first-degree relatives was obtained by a short questionnaire completed by both parents (53).
Anthropometric measurements and biochemical assays

Weight and height were measured using standard procedures (54). All participants were asked to refrain from intensive physical activity in the 3 days prior to the study. Fasting glucose was measured by glucose oxidase technique (Cobas Integra, Roche) and insulin by a chemiluminescent immunoassay method (ADVIA Centaur analyzer; Bayer Diagnostics).

Phenotypes

**Body mass index (BMI)** (kg/m²). Gender- and age-specific standard deviation scores (SDS) of BMI were calculated (Supplementary Figure 2) with the Growth Analyser RCT tool (version 3.0, https://www.growthanalyser.org/; Dutch Growth Research Foundation, Rotterdam, the Netherlands). Within the Growth Analyser, BMI data of 2-20 year-olds from Italy was used as the reference (54). We also dichotomised the BMI-SDS scores into obese (SDS≥2) and non-obese using two different definitions for the control groups: 1) non-obese (-2<BMI SDS<2), and 2) non-overweight (-1<BMI SDS<1). The distribution of the BMI SDS scores according to age of the participants is shown in Figure 5.

**Fasting insulin (FI) and glucose (FG).** None of the included children had diabetes according to WHO criteria (55). We used different units for FG and FI (mmol/l and pmol/l, respectively) for the calculation of indices. Indices of insulin sensitivity and beta cell function, namely HOMA-IR and HOMA-B were calculated using the HOMA calculator provided by the University of Oxford (https://www.dtu.ox.ac.uk/homacalculator/). To reduce skewness, FI, HOMA-IR and HOMA-B were natural logarithm transformed.
**DNA extraction**

DNA was extracted from 300ul of whole blood using the QIAsymphony DSP DNA kit. The extraction was performed on the automated extractor QIAsymphony SP workstation Qiagen, Hilden, Germany) according to manufacturer procedure. DNA was eluted in 200ul deionized water.

**SNP Genotyping**

For the analysis we selected 42 DNA variants reported in previous publications [Supplementary Table 1] (12, 28, 33, 56). The SNP genotyping was performed using Agena MassArray® System (Agena Bioscience, San Diego, USA). SNPs were assayed and typed using iPLEX® chemistry on a matrix assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometer. All biochemical reactions were performed as recommended by the manufacturer. The iPLEX single base extension were spotted on 384-SpectroChips and analysed in the MassARRAY Analyzer. MassARRAY Typer 4.0 software was used for evaluating and managing the genotype results.

**Quality control of genetic data.**

For the quality control (QC) purposes, missing rate per individuals and missing rate per SNP were calculated. In addition, for QC of SNP genotyping, positive and negative template control samples were included in each assay plate. Any assay found as positive in the negative template control was removed from the study. We kept SNPs with missingness ≤0.02, Hardy-Weinberg equilibrium test $p$-value<$1\times10^{-6}$, SNP
genotyping call rate≥95% and minor allele frequency (MAF)>1%.

**Statistical analyses**

All statistical analyses were performed using the software package R version 3.5.1 (57).

**Genetic Risk Scores (GRSs).** We calculated unweighted and weighted GRS for FG (20 SNPs), FI (5 SNPs) and T2D (36 SNPs) (see Supplementary Table 1 for the list of SNPs used). Effect sizes of genetic variants on each specific phenotype were obtained from large-scale consortia with mean age ranging from 31.0 to 73.4 (12, 28, 33, 56). For the unweighted GRS, the numbers of effect alleles (0,1,2) for each SNP were added up, while for the calculation of the weighted GRS, each effect allele count for each SNP was multiplied by the reported effect size of the effect allele (beta for all, i.e. log(OR) for T2D). If the reported SNP was not available in our data, we used a SNP in linkage disequilibrium and further weighted the SNP by the $r^2$ value (58). Both unweighted and weighted GRSs were further multiplied by the proportion of successfully genotyped SNPs per individual. The distributions of the GRSs for each respective set of established loci by phenotype were investigated against the distributions of the phenotypes of interest, e.g. FG, FI, and T2D. For the association analyses described below, the GRSs were standardised to have a mean value of 0 and standard deviation of 1 to allow comparison of effect estimates across different GRSs and the outcome variables.

**Association analysis.** We performed the association analysis with FG, FI, HOMA-B, HOMA-IR, and BMI SDS for the 42 genotyped SNPs assuming an additive genetic model using linear regression. Unweighted and weighted GRSs were also tested for association with FG, FI,
HOMA-B, HOMA-IR, and BMI SDS via linear regression. Logistic regression was used for the association analysis of GRS and obese vs. non-obese individuals. For the linear regression, we report the effects as regression coefficients with their 95% confidence intervals (CIs), whereas for logistic regression we provide estimates of odds ratios (OR) with their 95% CIs. We report unadjusted associations as well as analyses adjusted for 1) age, sex and BMI SDS (BMI adjustment not done when BMI or obesity is the outcome), and 2) age, sex, BMI SDS, and family history of T1D and T2D. We applied a Bonferroni correction to adjust for multiple testing. The \( P \)-value thresholds for statistical significance after Bonferroni correction were \( P=0.0025, 0.0014, \) and \( 0.008 \) for 5, 20, and 36 tests for FI, FG, and T2D SNPs, respectively.

**Mendelian Randomization.** We evaluated the casual relationship between FG/FI and BMI in a one-sample MR framework (Figure 6) using Two-Stage-Least Squares (2SLS) as implemented in the `ivreg(v.0.5-0)` R package. In 2SLS, the first regression model regresses the exposure on the genetic instruments providing fitted exposure values independent of the confounders. The second stage of 2SLS regresses the outcome on the fitted values of the exposure. The genetic instrument for FG was the same as the GRS for FG described previously, whereas the instrument for FI comprised of four variants after excluding the \( FTO \) variant from the FI GRS. The genetic instrument for BMI was constructed from the \( FTO \) and \( MC4R \) variants, and we used the effect sizes as reported (8). Since Locke et al. reported different variants for these two loci, we further weighted the effect sizes by the \( r^2 \) values between the reported lead variants and the variants used in the present study. We report F-statistics from the regression model of the exposure on the corresponding IV as a measure of instrument strength. Causal effects estimated in MR are only valid if the following core assumptions hold true: i) the genetic instrument has a true effect on the exposure and that ii) it only affects the outcome
through its effect on the exposure as well as iii) it is independent of any measured and unmeasured confounding factors of the exposure–outcome relationship.

**Contribution statement:** ZB, MK, AU, MM and IP wrote the manuscript and researched the data. MK, AU, AS performed the statistical analyses. MM, IP and BD designed the study and reviewed and edited the manuscript. RL, MB and LC contributed to data/sample collection and genotyping and reviewed and edited the manuscript. BD obtained funding for the study. All authors approved the final version of the manuscript. IP and MM 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.

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**Declaration of interest:** Authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

**Duality of interest:** The authors declare that there is no duality of interest associated with this manuscript.

**Data availability:** The datasets generated during and/or analysed during the current study are not publicly available for reasons related to privacy and participant consent but are available from the corresponding author on reasonable request.

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Legends to Figures
Figure 1 Relationships between the number of fasting glucose (FG) risk alleles and FG, HOMA-B and age- and gender standardised body mass index (BMI SDS).
Figure 2 Relationships between the number of fasting insulin (FI) risk alleles and FI, HOMA-IR and age- and gender standardised body mass index (BMI SDS).

Figure 3 Relationships between the number of type 2 diabetes (T2D) risk alleles and fasting glucose (FG), fasting insulin (FI), HOMA-B, HOMA-IR, and age- and gender standardised body mass index (BMI SDS).
Figure 4 Relationship between the number of fasting glucose (FG) risk alleles. The results from the association analyses using linear regression of both GRS types, weighted and unweighted, confirmed the findings from the comparison of phenotypic distributions in relation to allele counts (Table 2).
Figure 5 BMI SDS values of the Bambino Gesù study sample within their age distribution. Colours represent BMI SDS groups as displayed in the figure insert. The bars represent subject counts within each BMI SDS group by the age group (x axis). The green line shows the age density of the study sample.
Figure 6 Mendelian Randomization analysis to explore causality between BMI and FG/FI. (A) IV estimator is calculated as the beta coefficient from the association of GRS_{BMI} with FG or FI divided by the beta coefficient from the association of GRS_{BMI} with BMI (IV estimator = 0.31pmol/l/BMI unit). The IV estimator is equivalent to what is seen when FI is regressed on BMI. These results are supportive of a causal, non-confounded relationship. (B) The relationship of FG or FI with BMI.

Table 1 Characteristics of the study sample

| Phenotype | Males (N=889) | Females (N=771) | P-value for difference* |
|-----------|---------------|----------------|-------------------------|
| Age (years) | 9.07±3.74 | 9.12±3.91 | 0.82 |
| BMI (kg/m\(^2\)) | 20.83±5.59 | 20.59±5.47 | 0.37 |
| BMI SDS | 0.47±1.52 | 0.47±1.41 | 0.97 |
| FG (mmol/l) | 4.58±0.48 | 4.47±0.50 | 2.27×10\(^{-5}\) |
| FI (pmol/l) | 54.91±45.23 | 62.51±49.62 | 0.0012 |
| HOMA-B | 108.28±55.93 | 123.87±60.88 | 7.74×10\(^{-8}\) |
| HOMA-IR | 0.99±0.80 | 1.13±0.88 | 0.0013 |

*Difference between the mean values in male and female participants calculated by t-test.
Table 2  Association between the FG/ FI/ T2D genetic risk scores and unadjusted FG, FI, HOMA-B, HOMA-IR, and age- and gender-standardised BMI

| Model       | FG (mmol/l) | ln(FI (pmol/l)) | ln(HOMA-B) | ln(HOMA-IR) | BMI SDS |
|-------------|-------------|-----------------|------------|-------------|---------|
|             | β (95% CI)  | P-value         | β (95% CI) | P-value     | β (95% CI) | P-value |
| Unweighted GRS, unadjusted |             |                 |            |             |         |
| GRS_FG      | 0.075 (0.053, 0.097) | 1.58×10^{-11} | -0.011     | -0.041      | 5.13×10^{-8} | -0.0049 |
| GRS_FI      | 0.0023 (-0.020, 0.024) | 0.84          | 0.020      | 0.012       | 0.20     | 0.0081 |
| GRS_T2D     | 0.0074 (-0.015, 0.029) | 0.51          | -0.025     | -0.020 (-0.038, -0.0019) | 0.030 |
| Weighted GRS, unadjusted |             |                 |            |             |         |
| GRS_FG      | 0.082 (0.059, 0.111) | 1.010×10^{-11} | 0.013 (-0.025, 0.050) | 0.029 (-0.052, -0.0070) | 0.010 |
| GRS_FI      | 0.0016 (-0.022, 0.025) | 0.90          | 0.023 (-0.014, 0.060) | 0.014 (-0.0083, 0.037) | 0.22 |
| GRS_T2D     | 0.0064 (-0.018, 0.030) | 0.60          | -0.026 (-0.063, 0.011) | -0.020 (-0.042, 0.0027) | 0.085 |
| Unweighted GRS, adjusted |             |                 |            |             |         |
| GRS_FG      | 0.045 (0.023, 0.067) | 5.31×10^{-5}  | -0.034 (-0.050, 0.0029) | -0.036 (-0.054, -0.018) | 7.15×10^{-5} |
| GRS_FI      | 0.0011 (-0.021, 0.023) | 0.92          | -0.016 (-0.011, 0.042) | 0.0097 (-0.0082, 0.028) | 0.29 |
| GRS_T2D     | 0.018 (-0.0044, 0.040) | 0.42          | -0.013 (-0.040, 0.013) | -0.017 (-0.035, 0.0010) | 0.064 |
| Unweighted GRS, unadjusted |             |                 |            |             |         |
| GRS_FG      | 0.048 (0.025, 0.072) | 6.83×10^{-5}  | -0.017 (-0.054, 0.020) | -0.033 (-0.056, -0.011) | 0.0036 |

Abbreviations: BMI, body mass index; FG, fasting glucose; FI, fasting insulin; SDS, standard deviation score; T2D, type 2 diabetes.
Table 3. Effects of FG/ FI / T2D genetic risk scores on obesity

| Model  | Obese (N=205) vs. non-obese (N=1350) (control group 1) | Obese (N=205) vs. non-overweight (N=639) (control group 2) |
|--------|----------------------------------------------------------|-----------------------------------------------------------|
|        | Unadjusted | Adjusted | P-value | Unadjusted | Adjusted | P-value |
| Weighted GRS |
| GRS_FG | 1.00 (0.86-1.15) | 0.96 | 0.97 (0.83-1.13) | 0.74 | 1.00 (0.86-1.18) | 0.96 | 1.00 (0.84-1.19) | 0.99 |
| GRS_FI | 0.98 (0.85-1.13) | 0.78 | 0.98 (0.84-1.13) | 0.74 | 0.94 (0.81-1.10) | 0.48 | 0.93 (0.79-1.10) | 0.41 |
| GRS_T2D | 0.93 (0.80-1.07) | 0.31 | 0.91 (0.78-1.06) | 0.22 | 0.99 (0.85-1.15) | 0.89 | 0.99 (0.84-1.17) | 0.91 |
| Unweighted GRS |
| GRS_FG | 0.95 (0.82-1.10) | 0.48 | 0.93 (0.80-1.09) | 0.36 | 0.94 (0.30-1.03) | 0.45 | 0.93 (0.79-1.11) | 0.44 |
| GRS_FI | 0.99 (0.86-1.15) | 0.93 | 0.99 (0.85-1.15) | 0.86 | 0.96 (0.82-1.11) | 0.60 | 0.95 (0.80-1.12) | 0.52 |
| GRS_T2D | 0.97 (0.84-1.12) | 0.68 | 0.96 (0.82-1.12) | 0.58 | 1.02 (0.88-1.20) | 0.77 | 1.02 (0.86-1.20) | 0.83 |

Legend: FG, fasting glucose; FI, fasting insulin; GRS, genetic risk score; SDS, standard deviation score; T2D, type 2 diabetes.

Obese individuals: BMI SDS >=2; non-obese individuals: -2<BMI SDS<2.
Obese individuals: BMI SDS >=2; non-overweight individuals: -1 < BMI SDS < 1.

Adjusted for age, sex, and family history of T1D and T2D.

Table 4. Mendelian randomization analysis

| Causal relationship tested (exposure on outcome) | Epidemiological association | Causal effect, instrumental variable (IV) approach |
|-----------------------------------------------|----------------------------|-----------------------------------------------|
|                                               | β (95% CI) | P-value | F-stat (IV) | β_IV (95% CI) | P-value |
| FG on BMI                                     | 0.68 (0.54, 0.82) | 6.70×10^{-21} | 46.98 | 0.61 (-0.22, 1.43) | 0.15 |
| FI on BMI                                     | 0.96 (0.89, 1.043) | 1.75×10^{-109} | 0.79 | -2.55 (-11.15, 6.053) | 0.56 |
| BMI on FG                                     | 0.077 (0.061, 0.093) | 6.70×10^{-21} | 15.72 | 0.049 (-0.12, 0.21) | 0.56 |
| BMI on FI                                     | 0.27 (0.25, 0.29) | 1.75×10^{-109} | 15.72 | 0.31 (0.078, 0.54) | 0.0085 |

Mendelian Randomization analyses using the Two-Stage-Least Squares method. FG, fasting glucose (mmol/l); BMI, body mass index (kg/m^2); FI, fasting insulin (ln(pmol/l)). For the instrumental variable analyses, estimates are reported in units of the outcome as described in the previous sentence per amount of increase in the exposure attributable to one unit increase in the genetic instrument of the exposure. For the statistically significant causal relationship of BMI on FI, one unit increase in BMI GRS corresponds to 0.14 (95% CI [0.070;0.21]) standard deviation score increase in BMI.
Abbreviations

BMI – body mass index
BMI SDS – body mass index standard deviation score
CI – confidence interval
DESIR – Data from an Epidemiological Study on the Insulin Resistance Syndrome study
DNA – deoxyribonucleic acid
DPP – Diabetes Prevention Program
FG – fasting glucose
FI – fasting insulin
GRS – Genetic Risk Score
GWAS – genome-wide association studies
HOMA-B - homeostatic model assessment of beta cell function
HOMA-IR - homeostatic model assessment of insulin resistance
HUNT – Nord-Trøndelag Health Study
MAF – minor allele frequency
MAGIC – Meta-Analyses of Glucose and Insulin-related traits Consortium
MALDI-TOF – matrix assisted laser desorption/ionization time-of-flight
OR – odds ratio
QC – quality control
SD – standard deviation
SNP – single nucleotide polymorphism
T1D – type 1 diabetes
T2D – type 2 diabetes
TNF-α – tumour necrosis factor-alpha