Causal Associations in Type 2 Diabetes Development

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Context: Obesity, glucose, insulin resistance [homeostatic model assessment, version 2, for insulin resistance (HOMA2-IR)], and insulin secretion (HOMA2-β) have been associated with type 2 diabetes (T2D) observationally. However, the causal, genetic contribution of each parameter to this risk is largely unknown and important to study because observational data are prone to confounding but genetic, causal data are free of confounding and reverse causation.

Objective: We examined the causal, genetic contribution of body mass index (BMI), glucose level, C-peptide level, HOMA2-IR, and HOMA2-β to the risk of T2D in 95,540 individuals from the Copenhagen General Population Study and estimated the absolute 10-year risks.

Methods: Cox regression analysis, instrumental variable analysis, and Poisson regression analysis were performed to estimate the observational hazard ratios, causal, genetic ORs, and absolute 10-year risks of T2D.

Results: For 1-SD greater level, BMI was associated with an observational 66% (95% CI, 62% to 72%) and causal, genetic 121% (95% CI, 25% to 291%) greater risk of T2D; glucose with an observational 44% (95% CI, 41% to 46%) and causal, genetic 183% (95% CI, 56% to 416%) greater risk of T2D; and HOMA2-IR with an observational 30% (95% CI, 18% to 44%) and causal, genetic 12% (95% CI, 2% to 22%) greater risk of T2D. In contrast, for 1-SD greater level, HOMA2-β was associated with an observational 14% (95% CI, 11% to 16%) and causal, genetic 21% (95% CI, 8% to 32%) lower risk of T2D. The upper tertiles of HOMA2-IR were associated with absolute 10-year diabetes risks of 31% and 37% in obese women and men, age >60 years, and a glucose level of 6.1 to 11.0 mmol/L.

Conclusions: BMI, glucose level, HOMA2-IR, and HOMA2-β are causally associated with T2D. (***J Clin Endocrinol Metab*** **104: 1313–1324, 2019)**

The development of type 2 diabetes is characterized by a progressive deterioration of glucose tolerance over several years. Insulin is the key hormone for regulation of the plasma glucose level and, generally, normoglycemia is maintained by the balanced interplay between insulin sensitivity and insulin secretion. The normal pancreatic β-cell can adapt to changes in insulin sensitivity—thus, a decrease in insulin sensitivity will be accompanied by an upregulation of insulin secretion and *vice versa*. However, increasing age, physical inactivity, and weight gain are often accompanied by progressive insulin resistance, and, in some individuals, insulin production from pancreatic β-cells will become inadequate (1). Insulin resistance and insulin secretory dysfunction occur early during the development of type 2 diabetes and worsen as glucose tolerance further

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Abbreviations: CGPS, Copenhagen General Population Study; DIAGRAM, diabetes genetics replication and meta-analysis; GIANT, genetic investigation of anthropometric traits; HOMA2-β, homeostatic model assessment for insulin secretion; HOMA2-IR, homeostatic model assessment for insulin resistance; IVW, inverse variance-weighted; MAGIC, meta-analyses of glucose and insulin-related traits; MR-Egger, Mendelian randomization-Egger; WHO, World Health Organization.

deteriorates toward the development of diabetes. The plasma glucose level does not change markedly during the transition from normal glucose tolerance to impaired glucose tolerance but increases with further progression to diabetes. Thus, an increase in the plasma glucose level represents a relatively late event in the pathogenesis of type 2 diabetes (2). Nonetheless, diagnostic testing for type 2 diabetes has solely included a fasting or nonfasting plasma glucose measurement, a 2-hour glucose tolerance test, or testing of the related hemoglobin A1c level.

The incidence of type 2 diabetes is increasing worldwide, and ~46% of all type 2 diabetes cases remain undiagnosed (3). Redirecting the focus to the diagnosis of insulin resistance and insulin secretory dysfunction, rather than awaiting the state of hyperglycemia, with the aim of early intervention might reduce the incidence of type 2 diabetes and subsequent cardiovascular disease. Reluctance to intervene in preclinical stages of type 2 diabetes might have been intensified by the lack of proof of causal associations. To investigate causal associations, the Mendelian randomization approach using genetic variants with an effect on modifiable exposures of interest can be used. Because of the random assortment of genetic variants at conception, variants with an effect on the modifiable exposure are randomly distributed in relation to most potential confounders. Also, because the genetic variants are determined at conception and remain constant throughout life, the Mendelian randomization design will not be influenced by reverse causation (in which the outcome alters the exposure of interest). Thus, if, for example, high insulin resistance has a causal effect on type 2 diabetes risk, the genetic variants that increase insulin resistance lifelong would be expected to also increase the risk of disease. Instrumental variable analysis is the final step in the Mendelian randomization design, testing whether a causal association between the exposure of interest and the disease exists and uses altered levels of the exposure of interest according to the genetic variants. Thus, unlike an observational association, which can be highly confounded, can be influenced by reverse causation, and will not necessarily reflect a causal association, the genetic association will be unconfounded and free of reverse causation and can often infer causality (4, 5).

We previously found that two components of the metabolic syndrome (waist circumference and glucose level) were causally associated with type 2 diabetes (6). We have extended our search for causal determinants in type 2 diabetes. The aim of the present study was to explore whether the body mass index (BMI), glucose level, C-peptide level, insulin resistance [expressed by the homeostatic model assessment for insulin resistance (HOMA2-IR) index], and insulin secretory function [expressed by homeostatic model assessment for insulin secretion (HOMA2-β) percentage] have been causally associated with type 2 diabetes (7). Furthermore, we have provided absolute 10-year risks of type 2 diabetes that potentially could be used as a tool in the primary prevention of type 2 diabetes in the general population.

Materials and Methods

Study population

The Copenhagen General Population Study (CGPS) is a prospective study initiated in 2003 with ongoing inclusion (8). The participants were selected using the national Danish Civil Registration System to reflect the Danish population aged 20 to 100 years. At the examination, each participant completed a questionnaire, which was reviewed by an investigator present, and underwent a physical examination, with blood samples taken for biochemical analyses and DNA extraction. Individuals with type 2 diabetes at baseline were excluded from further analysis. The participants included in the analyses were censored at the event of type 2 diabetes, emigration, or death, whichever came first. The median follow-up time was 4.7 years (range, 0 to 8.6).

All participants were white and of Danish descent. The follow-up data were 100% complete (*i.e.*, we did not lose track of even one individual). The Herlev and Gentofte Hospital, Copenhagen University Hospital, and Danish ethical committees approved the present study (approval nos. KF-100.2039/91 and H-KF-01-144/01), which was conducted in accordance with the Declaration of Helsinki. All the participants provided written informed consent. We also used the publicly available data from 339,224 individuals from the GIANT (genetic investigation of anthropometric traits) consortium (available at: https://portals.broadinstitute.org/collaboration/giant/index.php/GIANT_consortium) (9), 46,186 individuals from the MAGIC (meta-analyses of glucose and insulin-related traits) consortium (available at: https://www.magicinvestigators.org/) (10), and 80,788 individuals from the DIAGRAM (diabetes genetics replication and meta-analysis) consortium (http://diagram-consortium.org/) (11).

Type 2 diabetes

We reviewed all hospital admissions and diagnoses entered in the national Danish Patient Registry and all causes of death in the national Danish Causes of Death Registry for information on a diagnosis of type 2 diabetes according to the World Health Organization (WHO), International Classification of Diseases, 8th edition code 250 and 10th edition codes E11, E13, and E14 was collected from 1977 through November 2014. A diagnosis of type 2 diabetes required a plasma glucose or hemoglobin A1c level according to the changing diagnostic criteria over time (12–14).

Covariates

Trained examiners measured the participants' body weight and height. The BMI was computed as the weight in kilograms divided by the height in square meters. The plasma levels of nonfasting glucose, triglycerides, and low-density lipoprotein cholesterol were measured using standard hospital assays for all participants. The plasma levels of nonfasting C-peptide were measured in a subset of 8255 consecutive individuals. Their blood pressure was measured using an automatic digital blood pressure monitor (Kivex) and adjusted for self-reported use of

antihypertensive medication by adding a constant value of 10 mm Hg for systolic blood pressure and 5 mm Hg for diastolic blood pressure (15). Statin use was self-reported. Alcohol consumption was recorded in U/wk (1 U = 12 g of alcohol). Smoking was recorded as ever smoker vs never smoker, and the number of pack-years was calculated for the ever smokers. The time since the last meal was recorded in hours. Physical activity was categorized from self-reported data as low, moderate, and high. Education was recorded as schooling for <10, 10 to 12, and ≥13 years. The participants' income was recorded as <100,000Dkr, 1 to 400,000Dkr, 4 to 600,000Dkr, and >600,000Dkr.

Assessment of insulin resistance and β-cell dysfunction

Insulin resistance was expressed as the HOMA2-IR index and β-cell function as the HOMA2-β percentage, calculated from paired values of nonfasting plasma levels of glucose and C-peptide using the HOMA2, released as computer HOMA Calculation software from the University of Oxford (16). The HOMA2 is determined from the fasting glucose and insulin/C-peptide levels. The HOMA2-IR and HOMA2-β measures used in the present study were determined from paired values of nonfasting glucose and C-peptide and, thus, were not standardized to steady state. The levels of glucose, C-peptide, HOMA2-IR, and HOMA2-β as a function of hours since the last meal are provided in an online repository (7).

Genetic variants

We genotyped variants in *FTO* (rs9939609), *MC4R* (rs17782313), *TMEM18* (rs6548238), *BDNF* (rs10767664), and *GNPDA2* (rs10938397), which influenced BMI in 96,867 participants, and *G6PC2* (rs560887), *DGKB* (rs2191349), *ADRA2A* (rs10885122), and *ADCY5* (rs11708067), which influenced glucose levels in 105,475 participants. In addition, *IRS1* (rs2943641) and *IRS1* (rs7578326) were found to influence the HOMA2-IR index in 97,841 participants, and *CDKN2A/B* (rs10811661), *CDKN2A/B* (rs2383206), *CDKN2A/B* (rs7776061), *JAZF1* (rs864745), and *TCF7L2* (rs7903146) were found to influence the C-peptide and HOMA2-β percentage levels in 93,815 participants (7). We selected the genotypes previously shown to have the largest effect on the components and without major pleiotropic effects. The genetic variants were genotyped using LGC Genomics (available at: http://www.lgcgroup.com/) and divided into three or four groups of appropriate sizes to ensure sufficient statistical power and with a reasonably large reference group representing the background population.

Statistical analysis

We used Stata SE, version 13.1 (StataCorp, College Station, TX). Deviation from the Hardy-Weinberg equilibrium was tested using the Pearson χ^2 test. For trend tests across ordered groups, using the Cuzick nonparametric extension of a Wilcoxon rank sum test, groups of participants classified by alleles associated with BMI, glucose and C-peptide levels, HOMA2-IR index, and HOMA2-β percentage were ranked according to the changing levels and coded as 0, 1, 2, 3, and so forth.

First, to test whether the BMI, glucose and C-peptide levels, HOMA2-IR index, and HOMA2-β percentage were observationally associated with type 2 diabetes risk, Kaplan-Meier curves were used to estimate the cumulative incidence. Cox regression models with age as the time scale and left truncation (delayed entry at examination) were used to estimate the hazard ratios for type 2 diabetes. The individuals were grouped according to the tertiles of BMI, glucose, C-peptide, and HOMA2-IR index, and the lowest tertile was used as the reference group. Considering that pancreatic β-cells have the ability to compensate for insulin resistance by increasing insulin release, the individuals were grouped according to the HOMA2-β percentage as <90%, 90% to 110%, and >110%, and the middle group (90% to 110% of normal) was used as the reference group. Adjustment was made for sex, age (as time scale), BMI, C-peptide level, HOMA2-IR index, HOMA2-β percentage, triglyceride level, low-density lipoprotein cholesterol level, hypertension, alcohol consumption, smoking, hours since the last meal, physical activity, education, and income. The covariate being studied was excluded from the adjustment.

Second, to test whether the genotypes were associated with the BMI, glucose level, C-peptide level, HOMA2-IR index, and HOMA2-β percentage, one-way ANOVA was used to compare the levels of the five components as a function of genotype. Because the C-peptide levels were only measured for a subset of 8255 individuals, we used this subset to determine the effect of genotype on C-peptide and imputed the levels for ~85,560 individuals according to genotype to be able to include all individuals with genotype data available.

Third, to test whether the genotypes were associated with type 2 diabetes, Cox regression analysis was used to estimate the risk of type 2 diabetes as a function of genotypes associated with BMI, glucose level, C-peptide level, HOMA2-IR index, and HOMA2-β percentage.

Fourth, instrumental variable analysis was performed using a two-stage least-squares regression approach with the Stata add-in *ivreg2* (StataCorp) to assess potential causal relationships between altered levels of the exposure covariates [*i.e.*, the five components (BMI, glucose, C-peptide, HOMA2-IR index, and HOMA2-β percentage)] and type 2 diabetes risk using genetic variants as instruments for each of the five components. Only if both the observational and the genetic, causal estimate were substantial and affected risk were in the same direction, the component was considered to have a causal effect. We used 1-SD greater level of each component to be able to compare the potential causal effects of the five components.

Fifth, to validate our results, two-sample Mendelian randomization analysis was performed using data from the GIANT, MAGIC, and DIAGRAM consortia (9–11). We included the same genetic variants used in the instrumental variable analysis performed in the CGPS (7). In the two-sample Mendelian randomization analysis using multiple genetic variants, the ratio estimates from each genetic variant can be averaged using an inverse variance-weighted (IVW) formula from the reported meta-analysis data to provide an overall causal estimate known as the IVW estimate. In addition to the IVW estimate, a Mendelian randomization-Egger (MR-Egger) estimate will also be calculated when performing a two-sample Mendelian randomization analysis. Rather than setting the intercept term to zero, the term is estimated as a part of the analysis. If the intercept term is exactly equal to zero, the MR-Egger estimate will equal the IVW estimate. If the average pleiotropic effect is zero (known as balanced pleiotropy), the IVW method will give a consistent estimate of the causal effect. Hence, testing the intercept from the MR-Egger analysis will provide an assessment of the validity of the instrumental variable assumptions, with a nonzero intercept indicating that the

IVW estimate is biased. The test of whether the intercept differs from zero is referred to as the MR-Egger intercept test (17). The results of the MR-Egger intercept tests have been reported in the text, and both the MR-Egger estimates and the IVW estimates are provided in an online repository (7).

Sixth, the associations between potentially confounding factors and BMI, glucose level, C-peptide level, HOMA2-IR index, HOMA2-β percentage, type 2 diabetes, and the genetic instruments combined into allele scores were examined. Finally, the absolute 10-year risks of type 2 diabetes stratified by tertiles of HOMA2-IR index, glucose level in two groups (<6.1 and 6.1 to 11.0 mmol/L), BMI in three groups (<25, 25 to 30, and >30 kg/m^2) (selected as the three factors associated with the greatest causal risk of type 2 diabetes), and age in three groups (<40, 40 to 60, and >60 years) were estimated with the use of the regression coefficients from a Poisson regression model for women and men separately. Absolute risks are presented as estimated incidence rates (number of events per 10 years) in percentages.

Results

The baseline characteristics of 95,540 individuals from the CGPS stratified by type 2 diabetes status are provided in an online repository (7). During a median of 4.7 years (range, 0 to 8.6) of follow-up, 825 women and 1005 men had developed type 2 diabetes. All genotypes were in Hardy-Weinberg equilibrium ($P > 0.05$) (7).

BMI, glucose, C-peptide, insulin resistance (HOMA2-IR index), and insulin secretion (HOMA2-β percentage), and observational risk of type 2 diabetes

The plasma glucose level, C-peptide level, HOMA2-IR index, and HOMA2-β percentage decreased as a function of time since the last meal (7); thus, all observational estimates were adjusted for the time since the last meal. The variation in plasma glucose, C-peptide, HOMA2-IR index, and HOMA2-β percentage explained by the time since the last meal was 3% for glucose, 5% for C-peptide, 5% for HOMA2-IR, and 2% for HOMA2-β percentage. In the total study population, 62% had had blood samples taken 0 to 3 hours after the last meal, 35% had had blood samples taken 4 to 6 hours after the last meal, and 3% had had blood samples taken >6 hours after the last meal.

The cumulative incidence of type 2 diabetes was greater among the individuals with greater levels of BMI, glucose, C-peptide, HOMA2-IR index, and HOMA2-β percentage compared with individuals with lower levels (7). The risk of type 2 diabetes increased stepwise with increasing levels of BMI, glucose, C-peptide, and HOMA2-IR index but not with an increasing HOMA2-β percentage after multivariable adjustment (Fig. 1). The multivariable-adjusted risk of type 2 diabetes for individuals in the greatest tertile compared with individuals in the lowest tertile was increased by 340% (95% CI, 270% to 420%) for BMI, 120% (95% CI, 90% to 150%) for glucose, 330% (95% CI, 150% to 650%) for C-peptide, and 400% (95% CI, 190% to 760%) for the HOMA2-IR index. Compared with individuals in the middle HOMA2-β percentage group, the multivariable-adjusted risk for individuals with the lowest HOMA2-β percentage was 40% (95% CI, 0% to 100%) greater and 20% (95% CI, 10% to 50%) lower for individuals with the greatest HOMA2-β percentage (Fig. 1).

Genetic allele scores and levels of BMI, glucose, C-peptide, HOMA2-IR index, and HOMA2-β percentage

For the genetic allele scores, 7 to 10 vs 0 to 4 BMI increasing alleles was associated with a 2.7% greater BMI; 6 to 8 vs 0 to 4 glucose increasing alleles with a 2.4% greater glucose level; 7 to 10 vs 0 to 3 C-peptide increasing alleles with a 3.2% greater C-peptide level; 3 to 4 vs 0 insulin resistance increasing alleles with a 5.7% greater HOMA2-IR index; and 9 to 10 vs 0 to 2 β-cell function increasing alleles was associated with a 3.6% greater HOMA2-β percentage (Fig. 2) (7).

Genetic allele scores and risk of type 2 diabetes

For the genetic allele scores, 7 to 10 vs 0 to 4 BMI increasing alleles was associated with a 19% (95% CI, 9% to 30%) greater risk of type 2 diabetes; 6 to 8 vs 0 to 4 glucose increasing alleles with a 19% (95% CI, 10% to 29%) greater risk; 3 to 4 vs 0 insulin resistance increasing alleles with a 30% (95% CI, 15% to 47%) greater risk; and 9 to 10 vs 0 to 2 β-cell function increasing alleles was associated with a 35% (95% CI, 12% to 51%) lower risk of type 2 diabetes. Finally, 7 to 10 vs 0 to 3 C-peptide increasing alleles was associated with an insignificant result (Fig. 2).

Observational and causal type 2 diabetes risk in the CGPS

For a 1-SD greater level, BMI was associated with an observational multivariable adjusted 66% (95% CI, 62% to 72%) and a causal, genetic 121% (95% CI, 25% to 291%) greater risk of type 2 diabetes (Fig. 3). The corresponding values for glucose were 44% (95% CI, 41% to 46%) observational and 183% (95% CI, 56% to 416%) causal, genetic and for HOMA2-IR index were 30% (95% CI, 18% to 44%) observational and 12% (95% CI, 2% to 22%) causal, genetic greater risk. In contrast, a 1-SD greater HOMA2-β percentage level was associated with an observational multivariable-adjusted 14% (95% CI, 11% to 16%) and a causal, genetic 21% (95% CI, 8% to 32%) lower risk of type 2 diabetes. A 1-SD greater C-peptide level was associated observationally with a 16% (95% CI, 5% to 29%) greater risk of

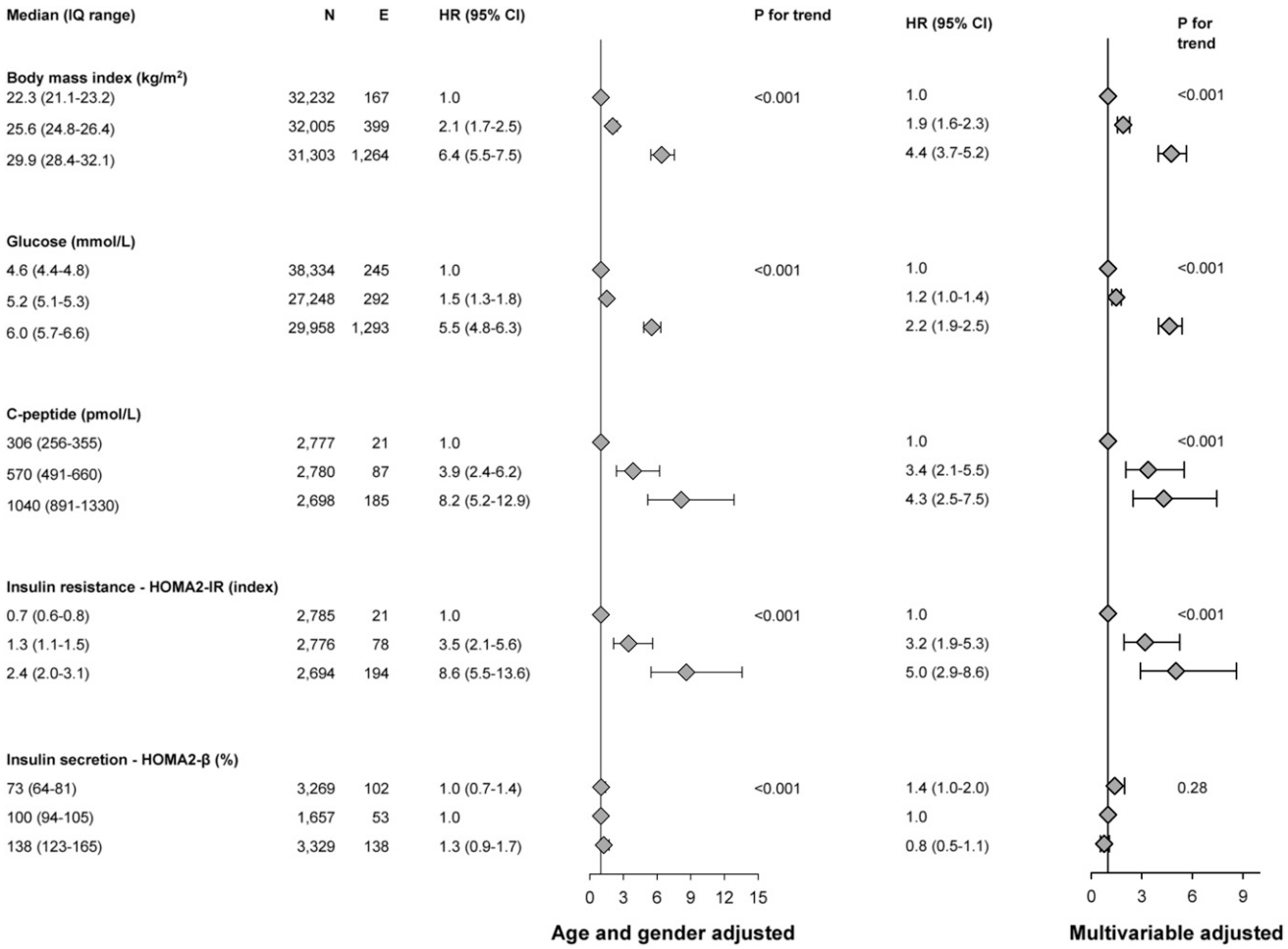

Figure 1. Risk of type 2 diabetes in the CGPS (n = 95,450) as a function of BMI, glucose level, C-peptide level, insulin resistance (calculated as HOMA2-IR index using HOMA2), and β-cell function (calculated as HOMA2-β percentage using HOMA2). Multivariable adjusted hazard ratios were adjusted for age (as the time scale), sex, hours since the last meal, physical activity, smoking, alcohol consumption, education, income, hypertension, triglyceride levels, low-density lipoprotein cholesterol levels, BMI, C-peptide levels, insulin resistance, and β-cell function. The covariate being studied was excluded from the adjustment. E, number of events; HR, hazard ratio; IQ, interquartile; N, number of participants.

type 2 diabetes; however, the genetic risk estimate was insignificant (Fig. 3).

Causal type 2 diabetes risk using data from the GIANT, MAGIC, and DIAGRAM consortia

The consortia data showed results similar to those from the data from the CGPS (7).

Associations with confounding factors

In the observational design, several of the potential confounders were associated with BMI, glucose, C-peptide, HOMA2-IR index, HOMA2-β percentage, and type 2 diabetes (7). In contrast, the genetic allele scores were not robustly associated with the potential confounders (7); that is, except with the factors they were expected to be associated with, such as the association of the BMI genotypes with hypertension and triglycerides (18, 19).

Absolute risk

The absolute 10-year risks of type 2 diabetes increased with an increasing HOMA2-IR index, from women to men, and with increasing BMI, glucose, and age (Fig. 4). In individuals with nonfasting glucose levels from 6.1 to 11.0 mmol/L, the highest absolute 10-year risks of type 2 diabetes of 31% and 37% were found in obese women and men aged >60 years and an HOMA2-IR index >1.7 (third tertile). We did not show results for individuals with nonfasting glucose levels >11.0 mmol/L because values greater than this level are diagnostic of type 2 diabetes. Consequently, information on the absolute 10-year risks is only relevant for individuals with nonfasting glucose levels less than the diagnostic threshold.

Discussion

The main findings of the present study of a general population cohort were that the levels of BMI, glucose, HOMA2-IR index, and HOMA2-β percentage contributed causally to the risk of type 2 diabetes.

We have previously reported that the glucose levels were causally associated with type 2 diabetes (6). Thus,

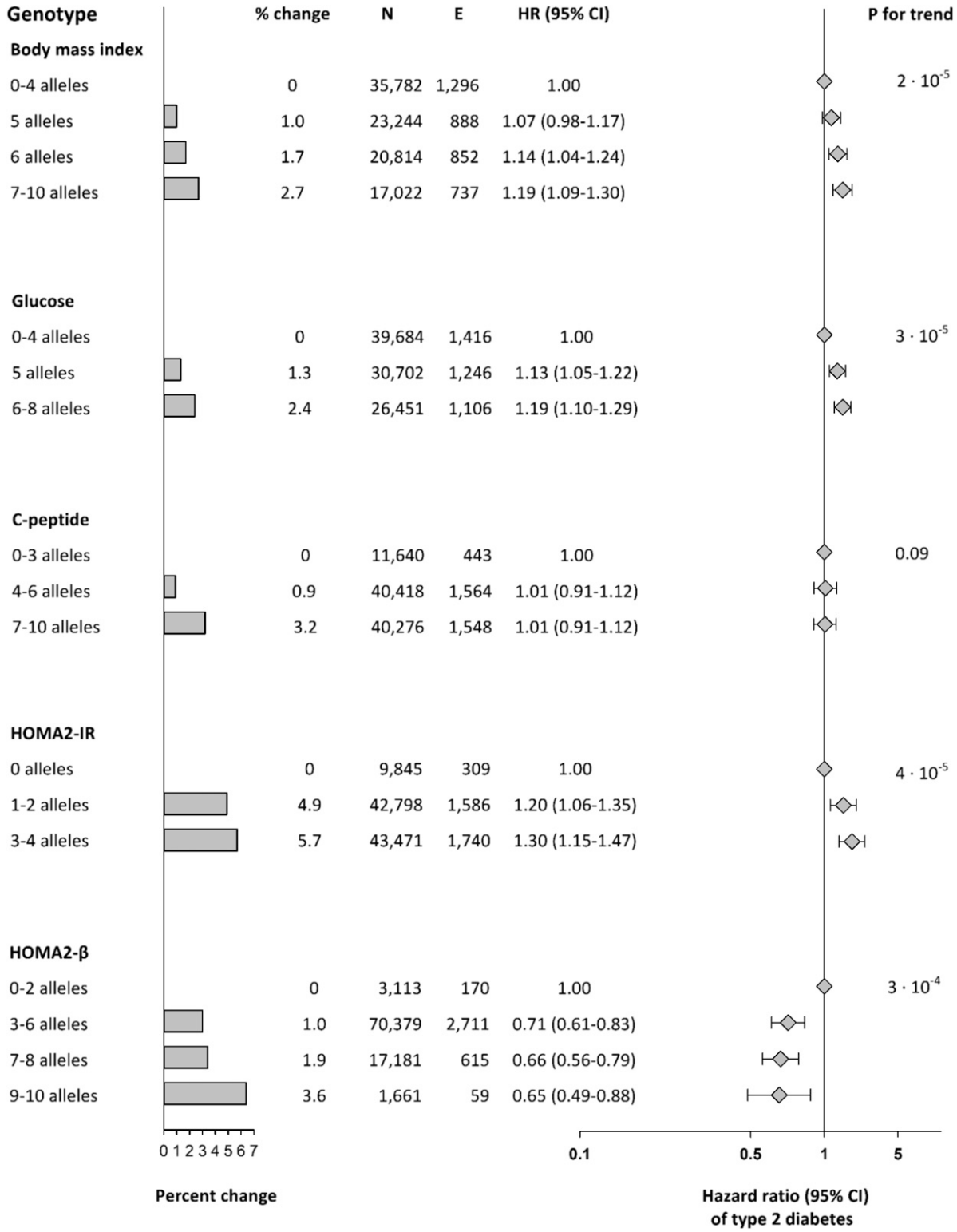

Figure 2. Genetic allele scores and levels of BMI, glucose, C-peptide, HOMA2-IR index, and HOMA2-β percentage and risk of type 2 diabetes. (Left) BMI, glucose, C-peptide, insulin resistance (calculated as HOMA2-IR index using HOMA2), and β-cell function (calculated as HOMA2-β percentage using HOMA2) as a function of genotypes selected as the genetic instrument representing each of the components in individuals from the CGPS. (Right) Risk of type 2 diabetes as a function of genotypes selected as the genetic instrument representing each of the components in individuals from the CGPS.

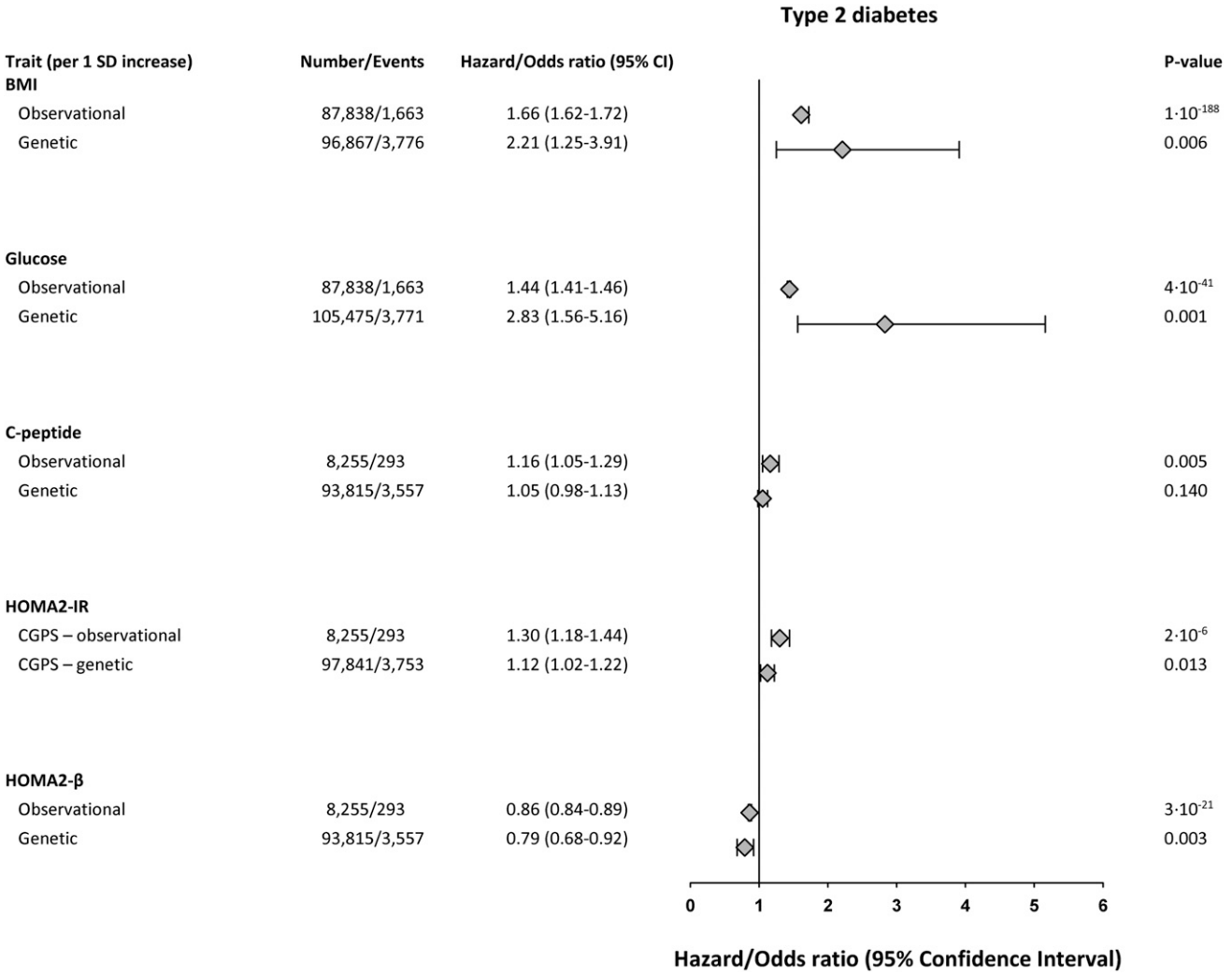

Figure 3. Using data from the CGPS, the observational and causal, genetic risk of type 2 diabetes for a 1-SD increase in BMI, glucose, C-peptide, insulin resistance (calculated as HOMA2-IR index using HOMA2), and β-cell function (calculated as HOMA2-β percentage using HOMA2) are given as multivariable adjusted hazard ratios and causal, genetic ORs with 95% CIs.

the finding of a causal, genetic association between the glucose level and type 2 diabetes was expected, in particular, because a diagnosis of type 2 diabetes is also defined by a high glucose level. We also previously found that waist circumference was causally associated with type 2 diabetes (6), in line with the present finding of the BMI having a causal effect on the risk of type 2 diabetes, also reported by others (20–23). Mechanistically, it also seems plausible that BMI is causally associated with the development of type 2 diabetes, because the mediators involved in the development of insulin resistance, such as nonesterified fatty acids, glycerol, hormones, cytokines, and proinflammatory substances, are all increased in obese individuals. Finally, insulin resistance with impairment of β-cell function will lead to the development of diabetes (24).

Our study included a northern European, white population and, therefore, might not necessarily be generalizable to other ethnic groups, especially for Asian populations who display relatively low BMIs (the BMI distribution is shifted to the left) in association with a high waist circumference or waist/hip ratio, reflecting elevated visceral obesity. This has led to the concern that application of the current WHO BMI cutoff points will underestimate obesity-related risks in Asian populations. Thus, a WHO expert consultation has suggested the following categories for many Asian populations: (i) $<$18.5 kg/m^2 as underweight; (ii) 18.5 to 23 kg/m^2 as an increasing but acceptable risk; (iii) 23 to 27.5 kg/m^2 as an increased risk; and (iv) $\geq$27.5 kg/m^2 as high risk (25). Also, the recommended waist circumference thresholds for abdominal obesity is 90 cm for men and 80 cm for women in Asian populations compared with 102 cm for men and 88 cm for women in European populations (26). In addition to anthropometry, ethnicity has been associated with factors that include genetic constitution, living conditions, and life style. Thus, differences in BMI distributions are likely not the only explanation for the

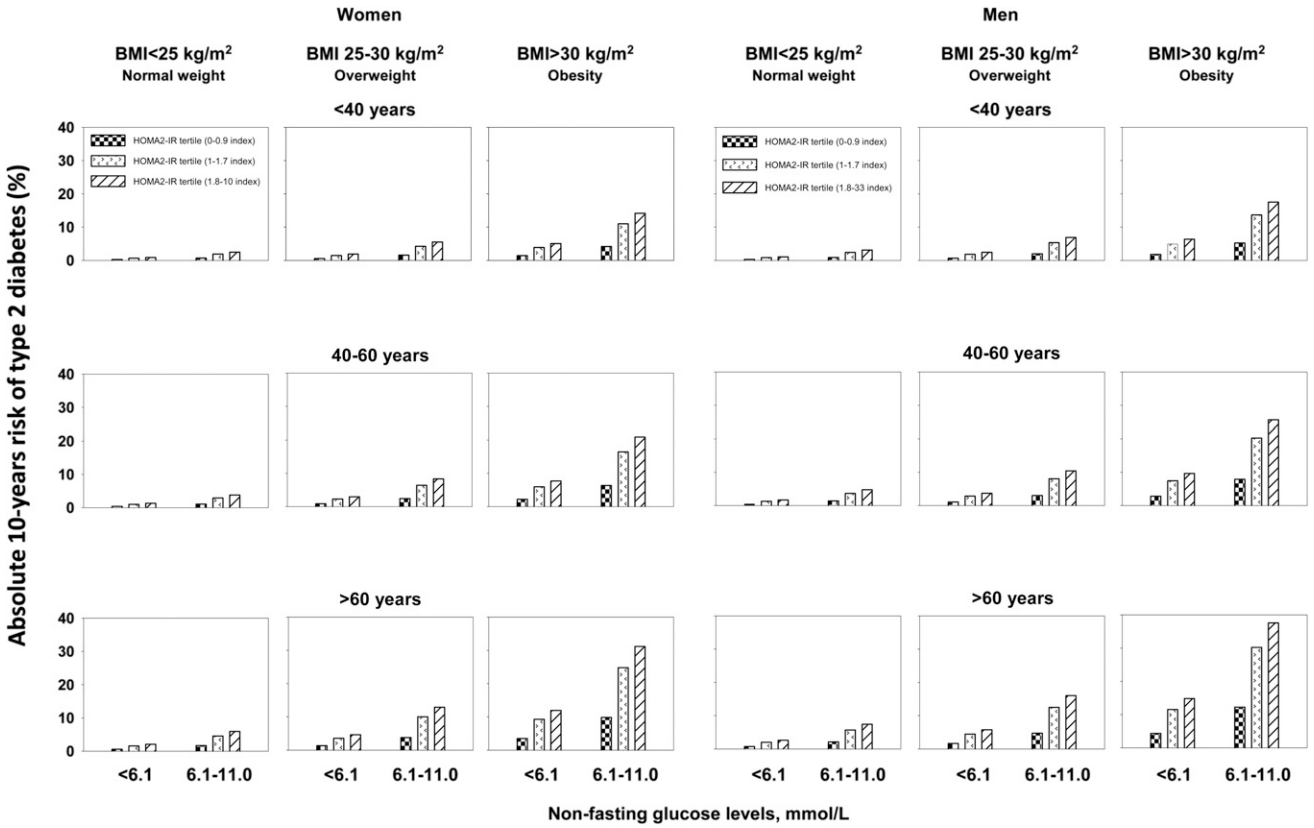

Figure 4. Absolute 10-y risks of type 2 diabetes stratified by tertiles of insulin resistance (calculated as HOMA2-IR index using HOMA2), sex, BMI, glucose level, and age for women and men separately. Checked bars indicate lower HOMA2-IR tertile for women and men (0 to 0.9 index). Arrow bars indicate middle HOMA2-IR tertile for women and men (1 to 1.7 index). Striped bars indicate upper HOMA2-IR tertile for women (1.8 to 10 index) and for men (1.8 to 33 index).

worldwide variation in the age-standardized prevalence of type 2 diabetes (27).

However, not all obese individuals will develop insulin resistant and, subsequently, type 2 diabetes. One explanation for this could be that the pancreatic β-cells of the islet of Langerhans release sufficient amounts of insulin to overcome the decreased insulin sensitivity (28). Another explanation could be that the expansion of adipose depots results from hyperplasia and hypertrophy of adipocytes in a depot-dependent fashion (29). A large individual variation in the size and expandability of different adipose tissue depots exists, and expansion of some depots has been associated with an increased risk of insulin resistance but the expansion of others has been associated with a decreased risk of insulin resistance (30). It has been established that adipose tissue acts as an endocrine organ (31). Subcutaneous adipose tissue and visceral adipose tissue have different endocrine functions, which might account for their varying associations with the risk of adverse outcomes. Visceral adipose tissue has been more inversely associated with levels of adiponectin, a protein hormone that modulates a number of metabolic processes, including glucose regulation and fatty acid oxidation, compared with subcutaneous adipose tissue (31, 32). Additionally, visceral adipose tissue releases greater levels of IL-6 and proteins involved in the fibrinolytic system, relative to subcutaneous adipose tissue, which also might account for visceral adipose tissue's stronger association with insulin resistance (31). An observational study found that for each 1-SD greater subcutaneous adipose tissue mass, the odds of insulin resistance was lower by 48% (33). In contrast, for a 1-SD greater visceral adipose tissue mass, the odds of insulin resistance was greater by 80% (33). Such findings suggest the existence of "benign" and "malign" obesity, although it is not known whether these patterns of regional fat distribution are the cause, or the consequence, of insulin resistance or, perhaps, are markers of related processes that determine both regional fat distribution and insulin resistance.

Several prospective studies have provided evidence that insulin resistance and insulin secretory dysfunction predict the development of type 2 diabetes in various populations (34–39). Our results support these findings, provide evidence of causal associations, and are in line with findings of a previous study, which also suggested causality (40). Some discussion has ensued regarding the relative importance of insulin resistance vs β-cell function in the development of type 2 diabetes (41). This discussion has resulted in part because the evaluation of

these parameters has frequently been performed in isolation in humans. Mechanistically, it seems plausible that both insulin resistance and β-cell dysfunction characterize the development of type 2 diabetes. The results from the present study have also indicated this possibility. Glucose homeostasis is vitally dependent on a feedback system incorporating the β-cell and insulin-sensitive tissues. The relationship is best described by a hyperbolic function such that any change in insulin sensitivity is balanced by a reciprocal and proportionate change in β-cell function (41). A study of healthy subjects showed that elevating their blood glucose levels for ≥20 hours led to enhanced β-cell function capacity and improved peripheral insulin uptake. These findings suggest that a genetic risk factor is necessary for the occurrence of β-cell function impairment (42).

The 10-year risk predictions can be used to advise individual patients. From our data, we could not define a cutpoint for the HOMA2-IR index, because our HOMA2-IR measurements should have been standardized and measured in different populations to establish a distribution from which a cutpoint could be drawn. However, in our study, we divided the levels of the HOMA2-IR index into tertiles, which makes the results independent of the HOMA2-IR index distribution. Thus, our results also apply to populations with a different distribution of HOMA2-IR index levels and suggest that the prediction of type 2 diabetes could be improved in both women and men by using the HOMA2-IR index for risk evaluation.

The strengths of our study included the size of the study population and the validity of the included type 2 diabetes cases requiring hospital contacts. Also, a key advantage of individual-level data, such as used in the present study compared with the reported published data for validation, is the ability to test the associations of the genetic instruments with a range of covariates in a systematic method. It is also reassuring that the results for BMI, HOMA2-IR index, and HOMA2-β percentage obtained using data from the GIANT, MAGIC, and DIAGRAM consortia with participants from many different European populations were similar to those for the CGPS, increasing the external validity of the results. The potential limitations of the present study consequently included that only inpatients and outpatients with type 2 diabetes were included in the present study, excluding all patients with type 2 diabetes only seen by general practitioners. The HOMA2-IR and HOMA2-β measures used in the present study were determined from paired values of the nonfasting glucose and C-peptide levels and, thus, were not standardized to a steady state. However, the HOMA values based on paired values of nonfasting glucose and C-peptide levels are largely equivalent to steady state (16, 43). Despite this limitation of the observational estimates, the causal, genetic estimates will not be biased by the nonfasting state. The potential limitations of the Mendelian randomization design include pleitropy, selection bias, and population stratification. However, using multiple genetic polymorphisms acting independently and via different pathways to change the levels of BMI, glucose, C-peptide, HOMA2-IR index, and HOMA2-β percentage, which might be informative in understanding the etiology of type 2 diabetes and might highlight specific mechanisms to prioritize for pharmacological intervention in all available individuals from an ethnically homogenous white population, these potential limitations are likely to have been avoided. Also, the genetic instruments were not robustly associated with the potential confounders, except for the factors they were expected to be associated with. Because insulin resistance often is related to obesity, which is a well-established risk factor for type 2 diabetes, we were especially pleased that our genetic HOMA2-IR index instrument was independent of the BMI, allowing us to study the isolated effect of insulin resistance on the risk of type 2 diabetes. However, genetic variants can be associated with a covariate without violating the instrumental variable assumptions (44).

In conclusion, BMI, glucose, HOMA2-IR index, and HOMA2-β percentage were all causally associated with type 2 diabetes risk. The results of our study have shown that the HOMA2-IR index could be used to identify individuals at type 2 diabetes risk in the preclinical stages.

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References

1. Stumvoll M, Goldstein BJ, van Haeften TW. Type 2 diabetes: principles of pathogenesis and therapy. *Lancet*. 2005;**365**(9467):1333–1346.
2. Weyer C, Bogardus C, Mott DM, Pratley RE. The natural history of insulin secretory dysfunction and insulin resistance in the pathogenesis of type 2 diabetes mellitus. *J Clin Invest*. 1999;**104**(6):787–794.
3. International Diabetes Federation. Diabetes Atlas, 6th ed. Brussels, Belgium: International Diabetes Federation; 2013.
4. Lawlor DA, Harbord RM, Sterne JA, Timpson N, Davey Smith G. Mendelian randomization: using genes as instruments for making

causal inferences in epidemiology. *Stat Med*. 2008;**27**(8):1133–1163.
5. Benn M, Nordestgaard BG. From genome-wide association studies to Mendelian randomization: novel opportunities for understanding cardiovascular disease causality, pathogenesis, prevention, and treatment. *Cardiovasc Res*. 2018;**114**(9):1192–1208.
6. Marott SC, Nordestgaard BG, Tybjærg-Hansen A, Benn M. Components of the metabolic syndrome and risk of type 2 diabetes. *J Clin Endocrinol Metab*. 2016;**101**(8):3212–3221.
7. Marott SCW, Nordestgaard BG, Tybjærg-Hansen A, Benn M. Data from: Causal associations in type 2 diabetes development. Figshare Digital Repository 2012. Deposited 5 November 2018. https://figshare.com/s/170ea62db334d5dc95bd.
8. Langsted A, Kamstrup PR, Benn M, Tybjærg-Hansen A, Nordestgaard BG. High lipoprotein(a) as a possible cause of clinical familial hypercholesterolaemia: a prospective cohort study. *Lancet Diabetes Endocrinol*. 2016;**4**(7):577–587.
9. Locke AE, Kahali B, Berndt SI, Justice AE, Pers TH, Day FR, Powell C, Vedantam S, Buchkovich ML, Yang J, Croteau-Chonka DC, Esko T, Fall T, Ferreira T, Gustafsson S, Kutalik Z, Luan J, Mägi R, Randall JC, Winkler TW, Wood AR, Workalemahu T, Faul JD, Smith JA, Zhao JH, Zhao W, Chen J, Fehrmann R, Hedman ÅK, Karjalainen J, Schmidt EM, Absher D, Amin N, Anderson D, Beekman M, Bolton JL, Bragg-Gresham JL, Buyske S, Demirkan A, Deng G, Ehret GB, Feenstra B, Feitosa MF, Fischer K, Goel A, Gong J, Jackson AU, Kanoni S, Kleber ME, Kristiansson K, Lim U, Lotay V, Mangino M, Leach IM, Medina-Gomez C, Medland SE, Nalls MA, Palmer CD, Pasko D, Pechlivanis S, Peters MJ, Prokopenko I, Shungin D, Stančáková A, Strawbridge RJ, Sung YJ, Tanaka T, Teumer A, Trompet S, van der Laan SW, van Setten J, Van Vliet-Ostaptchouk JV, Wang Z, Yengo L, Zhang W, Isaacs A, Albrecht E, Ärnlöv J, Arscott GM, Attwood AP, Bandinelli S, Barrett A, Bas IN, Bellis C, Bennett AJ, Berne C, Blagieva R, Blüher M, Böhringer S, Bonnycastle LL, Böttcher Y, Boyd HA, Bruinenberg M, Caspersen IH, Chen YI, Clarke R, Daw EW, de Craen AJM, Delgado G, Dimitriou M, Doney ASF, Eklund N, Estrada K, Eury E, Folkersen L, Fraser RM, Garcia ME, Geller F, Giedraitis V, Gigante B, Go AS, Golay A, Goodall AH, Gordon SD, Gorski M, Grabe HJ, Grallert H, Grammer TB, Gräßler J, Grönberg H, Groves CJ, Gusto G, Haessler J, Hall P, Haller T, Hallmans G, Hartman CA, Hassinen M, Hayward C, Heard-Costa NL, Helmer Q, Hengstenberg C, Holmen O, Hottenga JJ, James AL, Jeff JM, Johansson Å, Jolley J, Juliusdottir T, Kinnunen L, Koenig W, Koskenvuo M, Kratzer W, Laitinen J, Lamina C, Leander K, Lee NR, Lichtner P, Lind L, Lindström J, Lo KS, Lobbens S, Lorbeer R, Lu Y, Mach F, Magnusson PKE, Mahajan A, McArdle WL, McLachlan S, Menni C, Merger S, Mihailov E, Milani L, Moayyeri A, Monda KL, Morken MA, Mulas A, Müller G, Müller-Nurasyid M, Musk AW, Nagaraja R, Nöthen MM, Nolte IM, Pilz S, Rayner NW, Renstrom F, Rettig R, Ried JS, Ripke S, Robertson NR, Rose LM, Sanna S, Scharnagl H, Scholtens S, Schumacher FR, Scott WR, Seufferlein T, Shi J, Smith AV, Smolonska J, Stanton AV, Steinthorsdottir V, Stirrups K, Stringham HM, Sundström J, Swertz MA, Swift AJ, Syvänen AC, Tan ST, Tayo BO, Thorand B, Thorleifsson G, Tyrer JP, Uh HW, Vandenput L, Verhulst FC, Vermeulen SH, Verweij N, Vonk JM, Waite LL, Warren HR, Waterworth D, Weedon MN, Wilkens LR, Willenborg C, Wilsgaard T, Wojczynski MK, Wong A, Wright AF, Zhang Q, Brennan EP, Choi M, Dastani Z, Drong AW, Eriksson P, Franco-Cereceda A, Gådin JR, Gharavi AG, Goddard ME, Handsaker RE, Huang J, Karpe F, Kathiresan S, Keildson S, Kiryluk K, Kubo M, Lee JY, Liang L, Lifton RP, Ma B, McCarroll SA, McKnight AJ, Min JL, Moffatt MF, Montgomery GW, Murabito JM, Nicholson G, Nyholt DR, Okada Y, Perry JRB, Dorajoo R, Reinmaa E, Salem RM, Sandholm N, Scott RA, Stolk L, Takahashi A, Tanaka T, van 't Hooft FM, Vinkhuyzen AAE, Westra HJ, Zheng W, Zondervan KT, Heath AC, Arveiler D, Bakker SJL, Beilby J, Bergman RN, Blangero J, Bovet P, Campbell H, Caulfield MJ, Cesana G, Chakravarti A, Chasman DI, Chines PS, Collins FS, Crawford DC, Cupples LA, Cusi D, Danesh J, de Faire U, den Ruijter HM, Dominiczak AF, Erbel R, Erdmann J, Eriksson JG, Farrall M, Felix SB, Ferrannini E, Ferrières J, Ford I, Forouhi NG, Forrester T, Franco OH, Gansevoort RT, Gejman PV, Gieger C, Gottesman O, Gudnason V, Gyllensten U, Hall AS, Harris TB, Hattersley AT, Hicks AA, Hindorff LA, Hingorani AD, Hofman A, Homuth G, Hovingh GK, Humphries SE, Hunt SC, Hyppönen E, Illig T, Jacobs KB, Jarvelin MR, Jöckel KH, Johansen B, Jousilahti P, Jukema JW, Jula AM, Kaprio J, Kastelein JJP, Keinanen-Kiukaanniemi SM, Kiemeney LA, Knekt P, Kooner JS, Kooperberg C, Kovacs P, Kraja AT, Kumari M, Kuusisto J, Lakka TA, Langenberg C, Marchand LL, Lehtimäki T, Lyssenko V, Männistö S, Marette A, Matise TC, McKenzie CA, McKnight B, Moll FL, Morris AD, Morris AP, Murray JC, Nelis M, Ohlsson C, Oldehinkel AJ, Ong KK, Madden PAF, Pasterkamp G, Peden JF, Peters A, Postma DS, Pramstaller PP, Price JF, Qi L, Raitakari OT, Rankinen T, Rao DC, Rice TK, Ridker PM, Rioux JD, Ritchie MD, Rudan I, Salomaa V, Samani NJ, Saramies J, Sarzynski MA, Schunkert H, Schwarz PEH, Sever P, Shuldiner AR, Sinisalo J, Stolk RP, Strauch K, Tönjes A, Trégouët DA, Tremblay A, Tremoli E, Virtamo J, Vohl MC, Völker U, Waeber G, Willemsen G, Witteman JC, Zillikens MC, Adair LS, Amouyel P, Asselbergs FW, Assimes TL, Bochud M, Boehm BO, Boerwinkle E, Bornstein SR, Bottinger EP, Bouchard C, Cauchi S, Chambers JC, Chanock SJ, Cooper RS, de Bakker PIW, Dedoussis G, Ferrucci L, Franks PW, Froguel P, Groop LC, Haiman CA, Hamsten A, Hui J, Hunter DJ, Hveem K, Kaplan RC, Kivimaki M, Kuh D, Laakso M, Liu Y, Martin NG, März W, Melbye M, Metspalu A, Moebus S, Munroe PB, Njølstad I, Oostra BA, Palmer CNA, Pedersen NL, Perola M, Pérusse L, Peters U, Power C, Quertermous T, Rauramaa R, Rivadeneira F, Saaristo TE, Saleheen D, Sattar N, Schadt EE, Schlessinger D, Slagboom PE, Snieder H, Spector TD, Thorsteinsdottir U, Stumvoll M, Tuomilehto J, Uitterlinden AG, Uusitupa M, van der Harst P, Walker M, Wallaschofski H, Wareham NJ, Watkins H, Weir DR, Wichmann HE, Wilson JF, Zanen P, Borecki IB, Deloukas P, Fox CS, Heid IM, O'Connell JR, Strachan DP, Stefansson K, van Duijn CM, Abecasis GR, Franke L, Frayling TM, McCarthy MI, Visscher PM, Scherag A, Willer CJ, Boehnke M, Mohlke KL, Lindgren CM, Beckmann JS, Barroso I, North KE, Ingelsson E, Hirschhorn JN, Loos RJF, Speliotes EK; LifeLines Cohort Study; ADIPOGen Consortium; AGEN-BMI Working Group; CARDIOGRAMplusC4D Consortium; CKDGen Consortium; GLGCICBP MAGIC Investigators; MuTHER Consortium; MIGen Consortium; PAGE Consortium; ReproGen Consortium; GENIE Consortium; International Endogene Consortium. Genetic studies of body mass index yield new insights for obesity biology. *Nature*. 2015;**518**(7538):197–206.
10. Dupuis J, Langenberg C, Prokopenko I, Saxena R, Soranzo N, Jackson AU, Wheeler E, Glazer NL, Bouatia-Naji N, Gloyn AL, Lindgren CM, Mägi R, Morris AP, Randall J, Johnson T, Elliott P, Rybin D, Thorleifsson G, Steinthorsdottir V, Henneman P, Grallert H, Dehghan A, Hottenga JJ, Franklin CS, Navarro P, Song K, Goel A, Perry JR, Egan JM, Lajunen T, Grarup N, Sparsø T, Doney A, Voight BF, Stringham HM, Li M, Kanoni S, Shrader P, Cavalcanti-Proença C, Kumari M, Qi L, Timpson NJ, Gieger C, Zabena C, Rocheleau G, Ingelsson E, An P, O'Connell J, Luan J, Elliott A, McCarroll SA, Payne F, Roccasecca RM, Pattou F, Sethupathy P, Ardlie K, Ariyurek Y, Balkau B, Barter P, Beilby JP, Ben-Shlomo Y, Benediktsson R, Bennett AJ, Bergmann S, Bochud M, Boerwinkle E, Bonnefond A, Bonnycastle LL, Borch-Johnsen K, Böttcher Y, Brunner E, Bumpstead SJ, Charpentier G, Chen YD, Chines P, Clarke R, Coin LJ, Cooper MN, Cornelis M, Crawford G, Crisponi L, Day IN, de Geus EJ, Delplanque J, Dina C, Erdos MR, Fedson AC, Fischer-Rosinsky A, Forouhi NG, Fox CS, Frants R, Franzosi MG, Galan P, Goodarzi MO, Graessler J, Groves CJ, Grundy S, Gwilliam R, Gyllensten U, Hadjadj S, Hallmans G, Hammond N, Han X, Hartikainen AL, Hassanali N, Hayward C, Heath SC, Hercberg S, Herder C, Hicks AA, Hillman DR, Hingorani AD,

Hofman A, Hui J, Hung J, Isomaa B, Johnson PR, Jørgensen T, Jula A, Kaakinen M, Kaprio J, Kesaniemi YA, Kivimaki M, Knight B, Koskinen S, Kovacs P, Kyvik KO, Lathrop GM, Lawlor DA, Le Bacquer O, Lecoeur C, Li Y, Lyssenko V, Mahley R, Mangino M, Manning AK, Martínez-Larrad MT, McAteer JB, McCulloch LJ, McPherson R, Meisinger C, Melzer D, Meyre D, Mitchell BD, Morken MA, Mukherjee S, Naitza S, Narisu N, Neville MJ, Oostra BA, Orrù M, Pakyz R, Palmer CN, Paolisso G, Pattaro C, Pearson D, Peden JF, Pedersen NL, Perola M, Pfeiffer AF, Pichler I, Polasek O, Posthuma D, Potter SC, Pouta A, Province MA, Psaty BM, Rathmann W, Rayner NW, Rice K, Ripatti S, Rivadeneira F, Roden M, Rolandsson O, Sandbaek A, Sandhu M, Sanna S, Sayer AA, Scheet P, Scott LJ, Seedorf U, Sharp SJ, Shields B, Sigurethsson G, Sijbrands EJ, Silveira A, Simpson L, Singleton A, Smith NL, Sovio U, Swift A, Syddall H, Syvänen AC, Tanaka T, Thorand B, Tichet J, Tönjes A, Tuomi T, Uitterlinden AG, van Dijk KW, van Hoek M, Varma D, Visvikis-Siest S, Vitart V, Vogelzangs N, Waeber G, Wagner PJ, Walley A, Walters GB, Ward KL, Watkins H, Weedon MN, Wild SH, Willemsen G, Witteman JC, Yarnell JW, Zeggini E, Zelenika D, Zethelius B, Zhai G, Zhao JH, Zillikens MC, Borecki IB, Loos RJ, Meneton P, Magnusson PK, Nathan DM, Williams GH, Hattersley AT, Silander K, Salomaa V, Smith GD, Bornstein SR, Schwarz P, Spranger J, Karpe F, Shuldiner AR, Cooper C, Dedoussis GV, Serrano-Ríos M, Morris AD, Lind L, Palmer LJ, Hu FB, Franks PW, Ebrahim S, Marmot M, Kao WH, Pankow JS, Sampson MJ, Kuusisto J, Laakso M, Hansen T, Pedersen O, Pramstaller PP, Wichmann HE, Illig T, Rudan I, Wright AF, Stumvoll M, Campbell H, Wilson JF, Bergman RN, Buchanan TA, Collins FS, Mohlke KL, Tuomilehto J, Valle TT, Altshuler D, Rotter JI, Siscovick DS, Penninx BW, Boomsma DI, Deloukas P, Spector TD, Frayling TM, Ferrucci L, Kong A, Thorsteinsdottir U, Stefansson K, van Duijn CM, Aulchenko YS, Cao A, Scuteri A, Schlessinger D, Uda M, Ruokonen A, Jarvelin MR, Waterworth DM, Vollenweider P, Peltonen L, Mooser V, Abecasis GR, Wareham NJ, Sladek R, Froguel P, Watanabe RM, Meigs JB, Groop L, Boehnke M, McCarthy MI, Florez JC, Barroso I; DIAGRAM Consortium; GIANT Consortium; Global BPgen Consortium; Anders Hamsten on behalf of Procardis Consortium MAGIC Investigators. New genetic loci implicated in fasting glucose homeostasis and their impact on type 2 diabetes risk. *Nat Genet*. 2010;**42**(2):105–116.

11. Gaulton KJ, Ferreira T, Lee Y, Raimondo A, Mägi R, Reschen ME, Mahajan A, Locke A, Rayner NW, Robertson N, Scott RA, Prokopenko I, Scott LJ, Green T, Sparso T, Thuillier D, Yengo L, Grallert H, Wahl S, Frånberg M, Strawbridge RJ, Kestler H, Chheda H, Eisele L, Gustafsson S, Steinthorsdottir V, Thorleifsson G, Qi L, Karssen LC, van Leeuwen EM, Willems SM, Li M, Chen H, Fuchsberger C, Kwan P, Ma C, Linderman M, Lu Y, Thomsen SK, Rundle JK, Beer NL, van de Bunt M, Chalisey A, Kang HM, Voight BF, Abecasis GR, Almgren P, Baldassarre D, Balkau B, Benediktsson R, Blüher M, Boeing H, Bonnycastle LL, Bottinger EP, Burtt NP, Carey J, Charpentier G, Chines PS, Cornelis MC, Couper DJ, Crenshaw AT, van Dam RM, Doney AS, Dorkhan M, Edkins S, Eriksson JG, Esko T, Eury E, Fadista J, Flannick J, Fontanillas P, Fox C, Franks PW, Gertow K, Gieger C, Gigante B, Gottesman O, Grant GB, Grarup N, Groves CJ, Hassinen M, Have CT, Herder C, Holmen OL, Hreidarsson AB, Humphries SE, Hunter DJ, Jackson AU, Jonsson A, Jørgensen ME, Jørgensen T, Kao WH, Kerrison ND, Kinnunen L, Klopp N, Kong A, Kovacs P, Kraft P, Kravic J, Langford C, Leander K, Liang L, Lichtner P, Lindgren CM, Lindholm E, Linneberg A, Liu CT, Lobbens S, Luan J, Lyssenko V, Männistö S, McLeod O, Meyer J, Mihailov E, Mirza G, Mühleisen TW, Müller-Nurasyid M, Navarro C, Nöthen MM, Oskolkov NN, Owen KR, Palli D, Pechlivanis S, Peltonen L, Perry JR, Platou CG, Roden M, Ruderfer D, Rybin D, van der Schouw YT, Sennblad B, Sigurðsson G, Stančáková A, Steinbach G, Storm P, Strauch K, Stringham HM, Sun Q, Thorand B, Tikkanen E, Tonjes A, Trakalo J, Tremoli E, Tuomi T, Wennauer R, Wiltshire S, Wood AR, Zeggini E, Dunham I, Birney E, Pasquali L, Ferrer J, Loos RJ, Dupuis J, Florez JC, Boerwinkle E, Pankow JS, van Duijn C, Sijbrands E, Meigs JB, Hu FB, Thorsteinsdottir U, Stefansson K, Lakka TA, Rauramaa R, Stumvoll M, Pedersen NL, Lind L, Keinanen-Kiukaanniemi SM, Korpi-Hyövälti E, Saaristo TE, Saltevo J, Kuusisto J, Laakso M, Metspalu A, Erbel R, Jöcke KH, Moebus S, Ripatti S, Salomaa V, Ingelsson E, Boehm BO, Bergman RN, Collins FS, Mohlke KL, Koistinen H, Tuomilehto J, Hveem K, Njølstad I, Deloukas P, Donnelly PJ, Frayling TM, Hattersley AT, de Faire U, Hamsten A, Illig T, Peters A, Cauchi S, Sladek R, Froguel P, Hansen T, Pedersen O, Morris AD, Palmer CN, Kathiresan S, Melander O, Nilsson PM, Groop LC, Barroso I, Langenberg C, Wareham NJ, O'Callaghan CA, Gloyn AL, Altshuler D, Boehnke M, Teslovich TM, McCarthy MI, Morris AP; DIAbetes Genetics Replication And Meta-analysis (DIAGRAM) Consortium. Genetic fine mapping and genomic annotation defines causal mechanisms at type 2 diabetes susceptibility loci. *Nat Genet*. 2015;**47**(12):1415–1425.
12. World Health Organisation. Definition and Diagnosis of Diabetes Mellitus and Intermediate Hyperglycemia: Report of a WHO/IDF Consultation. Geneva, Switzerland: World Health Organisation; Brussels, Belgium: International Diabetes Federation; 2006.
13. Genuth S, Alberti KG, Bennett P, Buse J, Defronzo R, Kahn R, Kitzmiller J, Knowler WC, Lebovitz H, Lernmark A, Nathan D, Palmer J, Rizza R, Saudek C, Shaw J, Steffes M, Stern M, Tuomilehto J, Zimmet P; Expert Committee on the Diagnosis and Classification of Diabetes Mellitus. Follow-up report on the diagnosis of diabetes mellitus. *Diabetes Care*. 2003;**26**(11):3160–3167.
14. Alberti KG, Zimmet PZ. Definition, diagnosis and classification of diabetes mellitus and its complications. Part 1: diagnosis and classification of diabetes mellitus provisional report of a WHO consultation. *Diabet Med*. 1998;**15**(7):539–553.
15. Tobin MD, Sheehan NA, Scurrah KJ, Burton PR. Adjusting for treatment effects in studies of quantitative traits: antihypertensive therapy and systolic blood pressure. *Stat Med*. 2005;**24**(19): 2911–2935.
16. Levy JC, Matthews DR, Hermans MP. Correct homeostasis model assessment (HOMA) evaluation uses the computer program. *Diabetes Care*. 1998;**21**(12):2191–2192.
17. Burgess S, Thompson SG. Interpreting findings from Mendelian randomization using the MR-Egger method. *Eur J Epidemiol*. 2017;**32**(5):377–389.
18. Varbo A, Benn M, Smith GD, Timpson NJ, Tybjaerg-Hansen A, Nordestgaard BG. Remnant cholesterol, low-density lipoprotein cholesterol, and blood pressure as mediators from obesity to ischemic heart disease. *Circ Res*. 2015;**116**(4):665–673.
19. Timpson NJ, Harbord R, Davey Smith G, Zacho J, Tybjaerg-Hansen A, Nordestgaard BG. Does greater adiposity increase blood pressure and hypertension risk? Mendelian randomization using the FTO/MC4R genotype. *Hypertension*. 2009;**54**(1):84–90.
20. Nordestgaard AT, Thomsen M, Nordestgaard BG. Coffee intake and risk of obesity, metabolic syndrome and type 2 diabetes: a Mendelian randomization study. *Int J Epidemiol*. 2015;**44**(2):551–565.
21. Afzal S, Tybjærg-Hansen A, Jensen GB, Nordestgaard BG. Change in body mass index associated with lowest mortality in Denmark, 1976-2013. *JAMA*. 2016;**315**(18):1989–1996.
22. Fall T, Hägg S, Mägi R, Ploner A, Fischer K, Horikoshi M, Sarin AP, Thorleifsson G, Ladenvall C, Kals M, Kuningas M, Draisma HH, Ried JS, van Zuydam NR, Huikari V, Mangino M, Sonestedt E, Benyamin B, Nelson CP, Rivera NV, Kristiansson K, Shen HY, Havulinna AS, Dehghan A, Donnelly LA, Kaakinen M, Nuotio ML, Robertson N, de Bruijn RF, Ikram MA, Amin N, Balmforth AJ, Braund PS, Doney AS, Döring A, Elliott P, Esko T, Franco OH, Gretarsdottir S, Hartikainen AL, Heikkilä K, Herzig KH, Holm H, Hottenga JJ, Hyppönen E, Illig T, Isaacs A, Isomaa B, Karssen LC, Kettunen J, Koenig W, Kuulasmaa K, Laatikainen T, Laitinen J, Lindgren C, Lyssenko V, Läärä E, Rayner NW, Männistö S, Pouta A, Rathmann W, Rivadeneira F, Ruokonen A, Savolainen MJ,

Sijbrands EJ, Small KS, Smit JH, Steinthorsdottir V, Syvänen AC, Taanila A, Tobin MD, Uitterlinden AG, Willems SM, Willemsen G, Witteman J, Perola M, Evans A, Ferrières J, Virtamo J, Kee F, Tregouet DA, Arveiler D, Amouyel P, Ferrario MM, Brambilla P, Hall AS, Heath AC, Madden PA, Martin NG, Montgomery GW, Whitfield JB, Jula A, Knekt P, Oostra B, van Duijn CM, Penninx BW, Smith GD, Kaprio J, Samani NJ, Gieger C, Peters A, Wichmann HE, Boomsma DI, de Geus EJ, Tuomi T, Power C, Hammond CJ, Spector TD, Lind L, Orho-Melander M, Palmer CN, Morris AD, Groop L, Järvelin MR, Salomaa V, Vartiainen E, Hofman A, Ripatti S, Metspalu A, Thorsteinsdottir U, Stefansson K, Pedersen NL, McCarthy MI, Ingelsson E, Prokopenko I; European Network for Genetic and Genomic Epidemiology (ENGAGE) consortium. The role of adiposity in cardiometabolic traits: a Mendelian randomization analysis. *PLoS Med*. 2013;**10**(6):e1001474.

23. Holmes MV, Lange LA, Palmer T, Lanktree MB, North KE, Almoguera B, Buxbaum S, Chandrupatla HR, Elbers CC, Guo Y, Hoogeveen RC, Li J, Li YR, Swerdlow DI, Cushman M, Price TS, Curtis SP, Fornage M, Hakonarson H, Patel SR, Redline S, Siscovick DS, Tsai MY, Wilson JG, van der Schouw YT, FitzGerald GA, Hingorani AD, Casas JP, de Bakker PI, Rich SS, Schadt EE, Asselbergs FW, Reiner AP, Keating BJ. Causal effects of body mass index on cardiometabolic traits and events: a Mendelian randomization analysis. *Am J Hum Genet*. 2014;**94**(2):198–208.
24. Al-Goblan AS, Al-Alfi MA, Khan MZ. Mechanism linking diabetes mellitus and obesity. *Diabetes Metab Syndr Obes*. 2014;**7**:587–591.
25. WHO Expert Consultation. Appropriate body-mass index for Asian populations and its implications for policy and intervention strategies. *Lancet*. 2004;**363**(9403):157–163.
26. Alberti KG, Eckel RH, Grundy SM, Zimmet PZ, Cleeman JI, Donato KA, Fruchart JC, James WP, Loria CM, Smith SC Jr; International Diabetes Federation Task Force on Epidemiology and Prevention; National Heart, Lung, and Blood Institute; American Heart Association; World Heart Federation; International Atherosclerosis Society; International Association for the Study of Obesity. Harmonizing the metabolic syndrome: a joint interim statement of the International Diabetes Federation Task Force on Epidemiology and Prevention; National Heart, Lung, and Blood Institute; American Heart Association; World Heart Federation; International Atherosclerosis Society; and International Association for the Study of Obesity. *Circulation*. 2009;**120**(16):1640–1645.
27. Nakagami T, Qiao Q, Carstensen B, Nhr-Hansen C, Hu G, Tuomilehto J, Balkau B, Borch-Johnsen K; DECODE-DECODA Study Group. Age, body mass index and type 2 diabetes-associations modified by ethnicity. *Diabetologia*. 2003;**46**(8):1063–1070.
28. Røder ME, Porte D Jr, Schwartz RS, Kahn SE. Disproportionately elevated proinsulin levels reflect the degree of impaired B cell secretory capacity in patients with noninsulin-dependent diabetes mellitus. *J Clin Endocrinol Metab*. 1998;**83**(2):604–608.
29. Tchoukalova YD, Votruba SB, Tchkonia T, Giorgadze N, Kirkland JL, Jensen MD. Regional differences in cellular mechanisms of adipose tissue gain with overfeeding. *Proc Natl Acad Sci USA*. 2010;**107**(42):18226–18231.
30. Preis SR, Massaro JM, Robins SJ, Hoffmann U, Vasan RS, Irlbeck T, Meigs JB, Sutherland P, D'Agostino RB Sr, O'Donnell CJ, Fox CS. Abdominal subcutaneous and visceral adipose tissue and insulin resistance in the Framingham heart study. *Obesity (Silver Spring)*. 2010;**18**(11):2191–2198.
31. Kershaw EE, Flier JS. Adipose tissue as an endocrine organ. *J Clin Endocrinol Metab*. 2004;**89**(6):2548–2556.
32. Kwon K, Jung SH, Choi C, Park SH. Reciprocal association between visceral obesity and adiponectin: in healthy premenopausal women. *Int J Cardiol*. 2005;**101**(3):385–390.
33. McLaughlin T, Lamendola C, Liu A, Abbasi F. Preferential fat deposition in subcutaneous versus visceral depots is associated with insulin sensitivity. *J Clin Endocrinol Metab*. 2011;**96**(11):E1756–E1760.
34. Ferrannini E. Insulin resistance versus insulin deficiency in non-insulin-dependent diabetes mellitus: problems and prospects. *Endocr Rev*. 1998;**19**(4):477–490.
35. Gerich JE. The genetic basis of type 2 diabetes mellitus: impaired insulin secretion versus impaired insulin sensitivity. *Endocr Rev*. 1998;**19**(4):491–503.
36. Lillioja S, Mott DM, Spraul M, Ferraro R, Foley JE, Ravussin E, Knowler WC, Bennett PH, Bogardus C. Insulin resistance and insulin secretory dysfunction as precursors of non-insulin-dependent diabetes mellitus: prospective studies of Pima Indians. *N Engl J Med*. 1993;**329**(27):1988–1992.
37. Lyssenko V, Almgren P, Anevski D, Perfekt R, Lahti K, Nissén M, Isomaa B, Forsen B, Homström N, Saloranta C, Taskinen MR, Groop L, Tuomi T; Botnia Study Group. Predictors of and longitudinal changes in insulin sensitivity and secretion preceding onset of type 2 diabetes. *Diabetes*. 2005;**54**(1):166–174.
38. Saad MF, Knowler WC, Pettitt DJ, Nelson RG, Charles MA, Bennett PH. A two-step model for development of non-insulin-dependent diabetes. *Am J Med*. 1991;**90**(2):229–235.
39. Weyer C, Tataranni PA, Bogardus C, Pratley RE. Insulin resistance and insulin secretory dysfunction are independent predictors of worsening of glucose tolerance during each stage of type 2 diabetes development. *Diabetes Care*. 2001;**24**(1):89–94.
40. Scott RA, Fall T, Pasko D, Barker A, Sharp SJ, Arriola L, Balkau B, Barricarte A, Barroso I, Boeing H, Clavel-Chapelon F, Crowe FL, Dekker JM, Fagherazzi G, Ferrannini E, Forouhi NG, Franks PW, Gavrila D, Giedraitis V, Grioni S, Groop LC, Kaaks R, Key TJ, Kühn T, Lotta LA, Nilsson PM, Overvad K, Palli D, Panico S, Quirós JR, Rolandsson O, Roswall N, Sacerdote C, Sala N, Sánchez MJ, Schulze MB, Siddiq A, Slimani N, Sluijs I, Spijkerman AM, Tjonneland A, Tumino R, van der A DL, Yaghootkar H, McCarthy MI, Semple RK, Riboli E, Walker M, Ingelsson E, Frayling TM, Savage DB, Langenberg C, Wareham NJ; RISC Study Group; EPIC-InterAct Consortium. Common genetic variants highlight the role of insulin resistance and body fat distribution in type 2 diabetes, independent of obesity. *Diabetes*. 2014;**63**(12):4378–4387.
41. Kahn SE. The relative contributions of insulin resistance and beta-cell dysfunction to the pathophysiology of type 2 diabetes. *Diabetologia*. 2003;**46**(1):3–19.
42. Gallagher D, Heymsfield SB, Heo M, Jebb SA, Murgatroyd PR, Sakamoto Y. Healthy percentage body fat ranges: an approach for developing guidelines based on body mass index. *Am J Clin Nutr*. 2000;**72**(3):694–701.
43. Wallace TM, Levy JC, Matthews DR. Use and abuse of HOMA modeling. *Diabetes Care*. 2004;**27**(6):1487–1495.
44. Burgess S, Scott RA, Timpson NJ, Davey Smith G, Thompson SG; EPIC- InterAct Consortium. Using published data in Mendelian randomization: a blueprint for efficient identification of causal risk factors. *Eur J Epidemiol*. 2015;**30**(7):543–552.