Mendelian randomization analyses suggest a causal role for circulating GIP and IL-1RA levels in homeostatic model assessment-derived measures of β-cell function and insulin sensitivity in Africans without type 2 diabetes

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Abstract

Background In vitro and in vivo studies have shown that certain cytokines and hormones may play a role in the development and progression of type 2 diabetes (T2D). However, studies on their role in T2D in humans are scarce. We evaluated associations between 11 circulating cytokines and hormones with T2D among a population of sub-Saharan Africans and tested for causal relationships using Mendelian randomization (MR) analyses.

Methods We used logistic regression analysis adjusted for age, sex, body mass index, and recruitment country to regress levels of 11 cytokines and hormones (adipsin, leptin, visfatin, PAI-1, GIP, GLP-1, ghrelin, resistin, IL-6, IL-10, IL-1RA) on T2D among Ghanaians, Nigerians, and Kenyans from the Africa America Diabetes Mellitus study including 2276 individuals with T2D and 2790 non-T2D individuals. Similar linear regression models were fitted with homeostatic modelling assessments of insulin sensitivity (HOMA-S) and β-cell function (HOMA-B) as dependent variables among non-T2D individuals ($n = 2790$). We used 35 genetic variants previously associated with at least one of these 11 cytokines and hormones among non-T2D individuals as instrumental variables in univariable and multivariable MR analyses. Statistical significance was set at 0.0045 (0.05/11 cytokines and hormones).

Results Circulating GIP and IL-1RA levels were associated with T2D. Nine of the 11 cytokines and hormones (exceptions GLP-1 and IL-6) were associated with HOMA-S, HOMA-B, or both among non-T2D individuals. Two-stage least squares MR analysis provided evidence for a causal effect of GIP and IL-RA on HOMA-S and HOMA-B in multivariable analyses (GIP ~ HOMA-S $\beta = -0.67$, P-value $= 1.88 \times 10^{-6}$ and HOMA-B $\beta = 0.59$, P-value $= 1.88 \times 10^{-5}$; IL-1RA ~ HOMA-S $\beta = -0.51$, P-value $= 8.49 \times 10^{-5}$ and HOMA-B $\beta = 0.48$, P-value $= 5.71 \times 10^{-4}$). IL-RA was partly mediated via BMI (30-34%), but GIP was not. Inverse variance weighted MR analysis provided evidence for a causal effect of adipsin on T2D (multivariable OR $= 1.83$, P-value $= 9.79 \times 10^{-6}$), though these associations were not consistent in all sensitivity analyses.

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Conclusions The findings of this comprehensive MR analysis indicate that circulating GIP and IL-1RA levels are causal for reduced insulin sensitivity and increased β-cell function. GIP's effect being independent of BMI suggests that circulating levels of GIP could be a promising early biomarker for T2D risk. Our MR analyses do not provide conclusive evidence for a causal role of other circulating cytokines in T2D among sub-Saharan Africans.

Keywords Cytokines, Hormones, Type 2 diabetes, Sub-Saharan Africans, Mendelian randomization, Causal inference

Background

The global burden of type 2 diabetes (T2D) is high and rising and disproportionally affects African-ancestry populations. In the USA, African Americans have a 1.6 times higher prevalence of T2D than European Americans [1] and sub-Saharan African migrants in Europe are nearly three times more likely to have T2D compared with Europeans [2]. Furthermore, a steady rise in T2D prevalence is observed in sub-Saharan Africa [3].

The reasons for the disproportionate burden of T2D among African-ancestry populations are not completely understood. Inflammatory processes have been implicated in the development and progression of T2D [4]. Such an inflammatory state is characterized by increased circulating levels of pro-inflammatory cytokines and hormones and reduced levels of anti-inflammatory ones [5]. Cytokines and hormones relevant to T2D can be classified into different clusters based on their site of production, including those produced by adipose tissue (such as adipsin, leptin, visfatin, and plasminogen activator inhibitor-1(PAI-1)), those produced by the gut (such as glucose-dependent insulinotropic peptide (GIP), glucagon-like peptide-1 (GLP-1), and ghrelin), and those produced by immune cells (such as resistin, interleukin 6 (IL-6), interleukin 10 (IL-10), and interleukin 1 receptor antagonist (IL-1RA)). Most studies on these circulating cytokines and hormones have been performed in vitro and in vivo [6, 7]. The observational studies available have associated a limited number of cytokines and hormones with insulin sensitivity and T2D in diverse populations [8–11], including African Americans and West Africans [12–15]. However, these studies have focused on a handful of cytokines and hormones, such as adiponectin [8, 13], adipsin [9], PAI-1 [10], IL-6 [11, 12, 14, 15], IL-10 [12, 14], and IL-1RA [12, 14], while analyses on other potentially diabetes-related cytokines and hormones such as visfatin, GIP, GLP-1, and ghrelin are scarce. In addition, cross-sectional observational studies are unable to determine causality, i.e. whether circulating cytokine and hormone levels are a cause or consequence of T2D.

Mendelian randomization (MR) analysis leverages genetic variants as instrumental variables to improve causal inference in observational studies. Specifically, MR studies are less affected by limitations such as unmeasured confounding and reverse causation because of the random distribution of genotypes at conception. MR studies assessing causality for the relationship between cytokines and hormones with T2D are limited. Wang et al. found evidence for a causal effect of leptin levels on T2D in European-ancestry individuals [16], while for IL1-RA, no evidence for a causal effect on glycaemic traits was found [17]. MR studies assessing the effect of other cytokines on T2D are lacking and MR studies for any cytokine or hormone on T2D are absent in sub-Saharan African populations. As levels of diabetes-related cytokines and hormones have been found to differ in African-ancestry individuals compared with European-ancestry individuals [18–21], the causal effect of these cytokines and hormones on T2D risk could also differ. A better understanding of the role of cytokines and hormones in T2D among African-ancestry populations may have important preventive and therapeutic implications.

Here, we aimed to (1) evaluate the association between 11 circulating cytokines and hormones with T2D-related phenotypes, and (2) infer causality in these associations using MR analyses in a population of sub-Saharan Africans.

Methods

Study design and data sources

The Africa America Diabetes Mellitus (AADM) study is the longest-running genetic epidemiology study of T2D in sub-Saharan Africa. The study enrolled over 6000 sub-Saharan African adults aged 25 years and above with T2D and adults without T2D (non-T2D individuals) from university medical centres in Nigeria, Ghana, and Kenya. The study design and procedures have been described in detail elsewhere [22–24]. In brief, individuals with T2D were enrolled through major medical centres in three cities in Nigeria (Ibadan, Enugu, and Lagos), two cities in Ghana (Accra, and Kumasi), and in the city of Eldoret in Kenya. Non-T2D individuals were enrolled from surrounding communities of the various participating medical centres. If individuals in the community expressed interest in participating in the study, they were then invited to the study clinic where the formal process of obtaining informed consent took place. Yoruba and Igbo (Nigeria), Akan and Gaa-Adangbe (Ghana), and Luhya, Kikuyu, and Kalenjin (Kenya) were the most common ethnolinguistic groups among the study participants.

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Measurements

Demographic data including age and sex were obtained through structured questionnaires. Height and weight were measured in light clothing and body mass index (BMI) was calculated as weight/height^2 (kg/m^2).

Fasting serum and plasma samples were obtained by trained personnel. Fasting glucose concentration was measured in mg/dl using the enzymatic reference method with hexokinase on a Roche analyzer. Eleven circulating cytokines and hormones, namely adipsin, leptin, visfatin, PAI-1, GIP, GLP-1, ghrelin, resistin, IL-6, IL-10, and IL-1RA were measured using multiplex bead-based flow cytometric immunoassays according to the manufacturer's instructions (Bio-Plex Pro human diabetes: 10-plex, Cat#171A7001M and 2-plex, Cat#171A7002M, Bio-Rad, Inc., Hercules, CA, USA). These commercial kits measure the levels of cytokines and hormones reported to be involved in obesity and diabetes pathophysiology. Data were collected using Bio-Plex 200®System (Luminex Corporation, Austin, TX) equipped with Bio-Plex Manager™ Software (Bio-Rad, Inc., Hercules, CA, USA). IL-6, IL-10, and IL-1RA were analysed in a subset of participants using enzyme-linked immunosorbent assay (ELISA) (Quantikine ELISA, R&D Systems, Minneapolis, MN, USA).

T2D was defined using the American Diabetes Association (ADA) criteria, i.e. a fasting glucose of ≥ 126 mg/dl (≥ 7.0 mmol/L), or an oral glucose tolerance test (OGTT) 2-h post load value of ≥ 200 mg/dl (11.1 mmol/L) on more than one occasion, or the reported use of glucose-lowering medication as prescribed by a physician confirmed by review of clinical records. The updated Homeostatic Model Assessment (HOMA) was used through the University of Oxford HOMA2 calculator to estimate insulin sensitivity (HOMA-S) and β-cell function (HOMA-B). The updated HOMA model is a computer model that derives HOMA-S and HOMA-B as percentages of a normal reference population rather than linear approximations (available from: https://www.dtu.ox.ac.uk/homacalculator/). HOMA-S is the reciprocal of HOMA-IR (insulin resistance) as calculated using the linear set of equations. Both HOMA-S and HOMA-B were calculated for non-T2D individuals only as the measures are deemed not to be valid in the presence of T2D.

Association analyses with T2D and HOMA measures

A total of 5066 participants were available for association analysis who had at least one of the 11 cytokines and hormones measured as well as data on T2D status (Fig. 1). We used *G*power* to calculate a post hoc power of 92.6% for an OR of 1.1 and 99.9% to detect an OR of 1.2 at an alpha of 0.05 [25]. A total of 2790 non-T2D

individuals were available for the analyses with HOMA-S and HOMA-B. We performed complete-case analyses for all three outcomes, i.e. individuals with missing values on any variable in the model were excluded from the analyses for that specific outcome. An overview of all analyses performed can be found in Fig. 1.

Analyses were performed using the R statistical computing platform (version 4.2.2) and R studio (version 2022.12.0) [26, 27]. The *bestNormalize* R package was used to find the best-performing transformation for all cytokines, hormones, and both HOMA measures [28]. The ordered quantile (ORQ) transformation was found to be optimal and was applied to the cytokine/hormone and HOMA measures. We subsequently fitted linear regression models adjusted for age, sex, BMI, and recruitment country among non-T2D individuals to regress each of the cytokines and hormones on HOMA-S and HOMA-B. Logistic regression models were fitted to assess the association between each of the cytokines and hormones with T2D status with adjustment for age, sex, BMI, and recruitment country.

Instrument selection

For each cytokine and hormone, instruments were derived from previously published genome-wide association analyses among AADM study participants without T2D [29]. In brief, participants' samples were genotyped using the Affymetrix Axiom PANAFR SNP array or Illumina's Multi-Ethnic Global Array (MEGA) [24]. Quality control resulted in a sample-level genotype call rate of at least 0.95 for all samples. The SNP datasets were filtered for missingness per marker (> 0.05), minor allele frequency (< 0.01), and Hardy–Weinberg equilibrium (*P*-value ≤ 1×10^{-6}) and imputed using the African Genome Resources Haplotype Reference Panel via the Sanger Imputation Service. Genome-wide quantitative linear regression analyses were performed for all cytokines and hormones separately with adjustment for age, sex, T2D, the first three principal components (PCs), and a genetic relatedness matrix [29]. For instrument selection, we used these summary statistics from which we additionally filtered out SNPs with low imputation quality by excluding SNPs with INFO scores of < 0.8. All SNPs with a genome-wide *P*-value of < 5×10^{-7} were subsequently selected as potential instruments. We then grouped these SNPs into genetic loci using pairwise linkage disequilibrium (LD) analysis. This procedure, commonly known as "LD clumping" was performed separately for each cytokine and hormone. The PLINK 1.9 software [30] was used for this purpose, setting an r^2 threshold of < 0.1 and all unrelated AADM participants as the LD reference. The SNPs

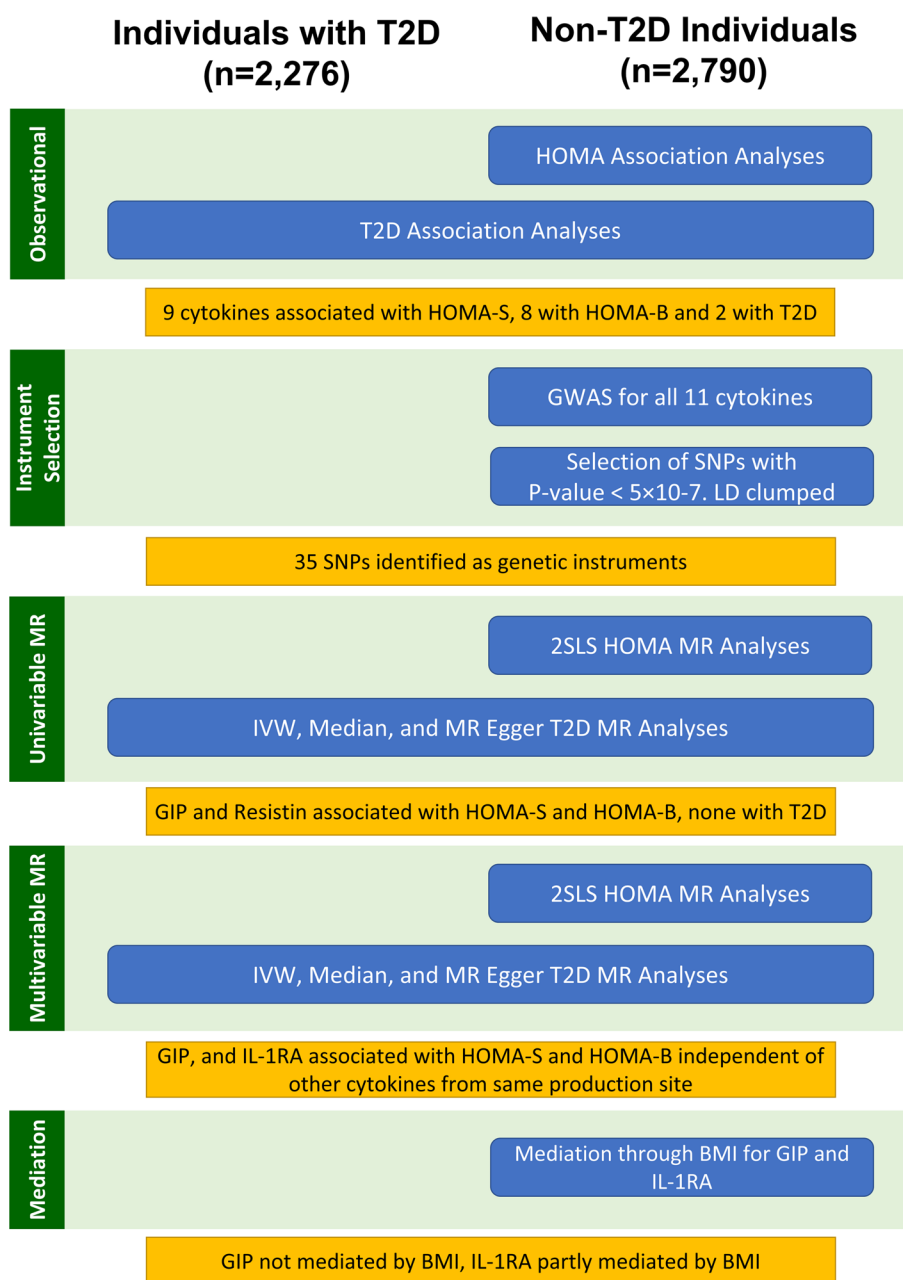Meeks *et al. Genome Medicine*   (2023) 15:108

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Fig. 1 Overview of analyses performed to identify circulating cytokines and hormones causally associated with HOMA-S, HOMA-B, and/or T2D. T2D = type 2 diabetes, HOMA = homeostatic modelling assessment of insulin sensitivity and insulin secretion, GWAS = genome-wide association study, LD = linkage disequilibrium, 2SLS = two-stage least squares, IVW = inverse variance weighted, MR = Mendelian randomization, BMI = body mass index

with the lowest *P*-value in each clump were selected as independent instruments. We used the *mRnd* online web tool to calculate the power of the MR analyses with these selected instruments for an α of 0.05 and a true causal effect size of $\beta = 0.2$ for the HOMA measures and OR = 1.5 for T2D [31].

Mendelian randomization analysis
Univariable Mendelian randomization analysis
To estimate the univariable effect of each of the cytokines and hormones on the continuous outcomes HOMA-S and HOMA-B, the two-stage least squares (2SLS) method was used through the ivreg function of the *AER*

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package [32]. In these analyses performed among non-T2D only ($n = 2790$), each cytokine was regressed on its genetic instruments. The fitted values of these regressions were subsequently used in regression with HOMA-S and HOMA-B as dependent variables.

For the univariable T2D MR analysis ($n = 5066$), the inverse variance weighted (IVW) method as implemented in the *MendelianRandomization* R package was used for multi-SNP instruments, while for single-SNP instruments (i.e. IL-6) the Wald ratio was used [33]. We calculated the regression coefficients for the instrument-cytokine/hormone associations in non-T2D individuals using linear regression and the odds ratios (ORs) with corresponding 95% confidence intervals (95% CI) for the instrument-T2D association using logistic regression analysis. This approach was chosen to reduce bias as it has been shown that when instruments are selected in the controls only for a binary outcome, bias follows the same pattern as in a two-sample setting [34]. The IVW method assumes that all genetic variants are valid instruments. We performed several sensitivity analyses using methods that make alternative assumptions to minimize risk of violating a core MR assumption. We used a random effects model for the IVW analyses in addition to the default fixed effects model. The random effects model allows for heterogeneity between the causal estimates by allowing over-dispersion in the model. We also report Cochran's Q and the I^2 statistic for the IVW analyses as an assessment of heterogeneity. In addition, we performed MR-Egger and weighted median analyses. These analyses could only be performed for cytokines and hormones with three or more genetic instruments. MR-Egger corrects for pleiotropy by assuming that the pleiotropic effects of each instrument follow a normal distribution with a mean of zero and a variance that can be estimated from the data [35]. MR-Egger allows for all genetic instruments to have pleiotropic effects but has much lower power than other methods. We report the MR-Egger intercept as a measure for the presence of horizontal pleiotropy. The weighted median method can handle up to 50% of invalid instruments or pleiotropic instruments, as long as the majority of the instruments are valid [36]. We considered concordance across MR methods as robust evidence for causality.

Multivariable Mendelian randomization analysis

Several of the eleven circulating cytokines and hormones are biologically related and have shared genetic predictors. To address this situation, we performed multivariable MR analysis in addition to the univariable MR analysis. For the multivariable MR analysis, we considered three clusters of cytokines and hormones: those produced by adipose tissue (adipsin, leptin, visfatin, and PAI-1), those produced by the gut (GIP, GLP-1, and ghrelin), and those produced by immune cells (resistin, IL-6, IL-10, and IL-1RA). While univariable MR analysis estimates the total (i.e. indirect and direct) effect of each cytokine on the outcomes, multivariable MR estimates the direct effect of each cytokine on the outcomes.

Multivariable 2SLS analyses for HOMA-S and HOMA-B were also performed using the *AER* R package, which first regressed the cytokines and hormones per group (adipose, gut, immune) on the genetic variants in a multivariate multiple linear regression. In the second stage, the HOMA outcome was regressed linearly on the fitted values of each of the cytokines and hormones. We additionally performed causal mediation analysis using the *mediation* R package on any statistically significant cytokines or hormone to examine whether their effect on HOMA-S and HOMA-B is mediated through BMI [37].

We used the mr_mvivw function from the *MendelianRandomization* R package to perform multivariable MR for the T2D outcome via the IVW method using a fixed effects model. We performed similar sensitivity analyses as for the univariable MR analyses: the IVW method using a random effects model, the multivariable MR-Egger method, and the multivariable median-based method.

Results

Characteristics of the study population

Out of the total 5066 individuals, 2790 were non-T2D individuals and 2276 were affected by T2D (Table 1). As expected, those affected by T2D were older on average and had a higher mean BMI. Median levels of the circulating cytokines and hormones were higher in those with T2D compared with non-T2D individuals for all cytokines and hormones except resistin, which had similar levels between T2D and non-T2D individuals, and IL-10, which had lower levels in those with T2D. This is expected for IL-10 given its anti-inflammatory properties.

Association analysis of cytokines and hormones with T2D and HOMA measures

Most cytokines and hormones were associated with the HOMA measures, the proxies for the hallmarks of T2D, insulin sensitivity (HOMA-S), and β-cell function (HOMA-B). All cytokines and hormones except for GLP-1 and IL-6 were associated with HOMA-S (Fig. 2A, Table 2). Resistin and IL-10 had a positive association with HOMA-S, while other cytokines and hormones had an inverse association with HOMA-S. GLP-1, IL-6, and IL-10 were not associated with HOMA-B at a Bonferroni-corrected *P*-value of 0.0045 (0.05/ 11 cytokines and hormones). The reverse of HOMA-S was seen for

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Table 1 Characteristics of the study population

	Total *N*	Non-T2D individuals (*N* = 2790)	Individuals with T2D (*N* = 2276)
Covariates			
Age (years)	5066	45.6 ± 15.4	56.2 ± 11.1
Sex (female)	5066	1674 (60.0%)	1401 (61.6%)
BMI (kg/m^2)	5066	26.4 ± 6.0	27.7 ± 5.5
Site	5066		
Ghana		769 (27.6%)	592 (26.0%)
Nigeria		1656 (59.4%)	1274 (56.0%)
Kenya		365 (13.1%)	410 (18.0%)
Cytokines and hormones			
Adipsin (ng/ml)	4854	1067.2 (837.2–1584.6)	1207.6 (887.0–1967.4)
Leptin (ng/ml)	4914	3.5 (0.67–10.8)	4.3 (1.5–10.4)
Visfatin (ng/ml)	4853	2.3 (1.4–4.0)	2.7 (1.6–4.4)
PAI-1 (ng/ml)	4914	31.2 (22.6–44.8)	33.6 (23.9–49.9)
GIP (pg/ml)	4956	183.3 (123.4–285.0)	252.9 (163.1–425.8)
GLP-1 (pg/ml)	4913	221.4 (177.0–282.6)	236.8 (183.0–317.7)
Ghrelin (pg/ml)	4923	248.6 (143.1–508.0)	311.2 (159.6–630.8)
Resistin (ng/ml)	4894	5.0 (3.3–7.7)	5.0 (3.3–8.1)
IL-6 (pg/ml)	1330	1.06 (0.73–1.67)	10.5 (0.69–1.70)
IL-10 (pg/ml)	1095	10.1 (8.3–12.7)	8.0 (7.0–11.0)
IL-1RA (pg/ml)	1416	301.1 (219.5–428.3)	351.4 (254.9–519.3)
Diabetes-related phenotypes			
HOMA-S	2552	136.6 (78.3–248.9)	*NA*
HOMA-B	2552	90.2 (60.9–130.9)	*NA*
Glucose (mmol/L)	4994	4.7 (4.1–5.1)	8.2 (5.9–12.4)

Continuous variables are in means ± SD for normally distributed variables. Non-normally distributed variables are expressed in medians and (25th–75th percentile). Categorical variables are in *n* (percentages). HOMA-S and HOMA-B were only calculated for non-T2D individuals

BMI body mass index, *T2D* type 2 diabetes

HOMA-B, with inverse associations for IL-10 and resistin and positive associations for all other cytokines and hormones.

Two out of the eleven cytokines and hormones were associated with T2D, namely GIP and IL-1RA (Fig. 2A, Table 2). One unit increase in ORQ transformed GIP levels was associated with 1.56 times higher odds for T2D (*P*-value = 6.37×10^{-38}) and one unit higher levels of ORQ transformed IL-1RA was associated with 1.34 higher odds (*P*-value = 1.03×10^{-5}).

Mendelian randomization analysis

A total of 35 SNPs were identified as genetic instruments for the eleven cytokines and hormones (Additional file 1; Table S1). All cytokines and hormones had multi-SNP instruments, except for IL-6 (Table 3). The three core assumptions of instrumental variables in MR analyses are the relevance (instrument is associated with exposure), exchangeability (instrument is not associated with confounders), and exclusion restriction (instrument only influences outcome via exposure) assumptions. We calculated the F-statistic and adjusted R squared

per instrument to evaluate the relevance assumption (Table 3). The cumulative F-statistic of the instruments of each of the cytokines and hormones ranged from 13.6 (leptin) to 74.8 (adipsin) which are all above the threshold of an F-statistic of 10 or higher to be considered acceptable for MR analysis. While the assumptions of exchangeability and exclusion restriction cannot be empirically tested, we undertook several approaches to minimize the risk of pleiotropy violating these assumptions. We tested for evidence of potential pleiotropy by regressing all genetic instruments on age, sex, and BMI in our dataset of sub-Saharan Africans. Three out of the 35 genetic instruments (rs80117394 [adipsin], rs854781 [adipsin], and rs146197730 [leptin]) were found to be associated with age, sex, or BMI at a nominal *P*-value of < 0.01 and therefore potentially pleiotropic. While including these potentially pleiotropic variants could bias the MR estimates, excluding them could result in a significant loss of power. We therefore conducted a sensitivity analysis that included only those instruments not associated with any of the tested confounders at a nominal *P*-value of < 0.01, as described in the guidelines for performing MR by
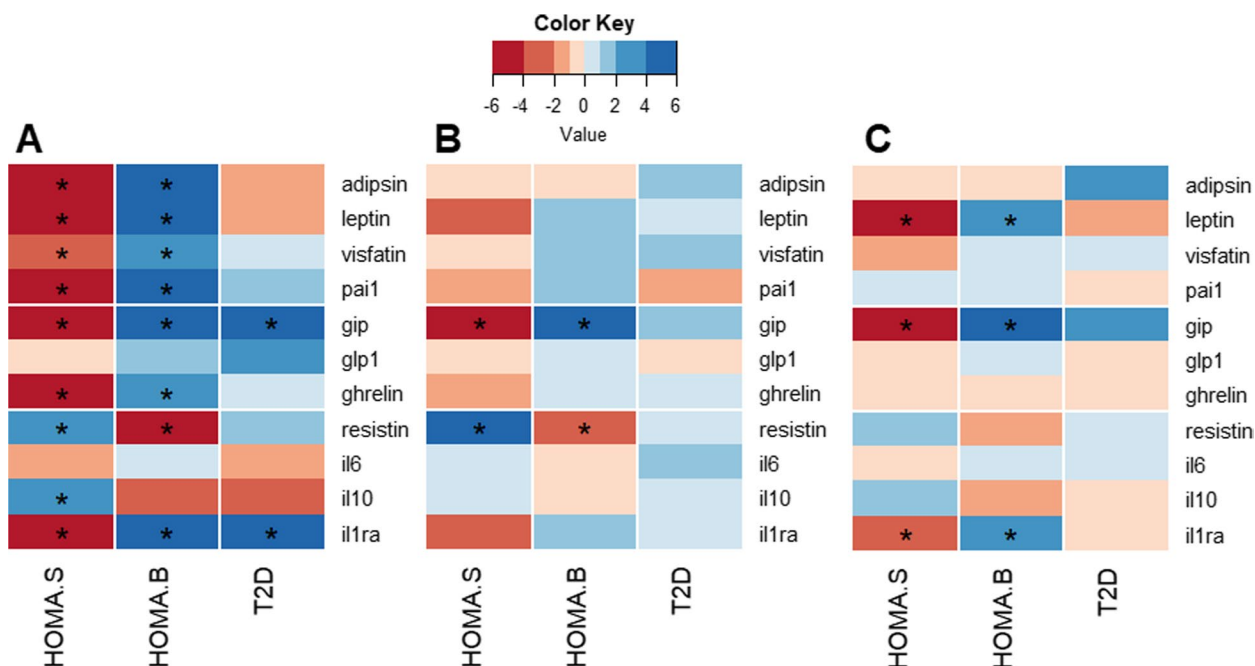
Fig. 2 Heat map of **A** associations, **B** univariable MR causal associations, and **C** multivariable MR causal associations. The colour gradient reflects negative (red) and positive (blue) *Z* values for T2D and *T* values for the HOMA measures. HOMA analyses were performed in non-T2D individuals only. For T2D in panels **B** and **C**, the estimates from the inverse variance weighted (IVW) random effects model are shown. An asterisk indicates statistical significance at a Bonferroni-corrected *P*-value of < 0.0045 (= 0.05/11 cytokines and hormones)

Table 2 Associations of circulating cytokines and hormones with insulin sensitivity (HOMA-S), β-cell function (HOMA-B) and T2D

Cytokine/hormone	HOMA-S		HOMA-B		T2D	
	β (95% CI)	*P*-value	*β* (95% CI)	*P*-value	OR (95% CI)	*P*-value
Adipsin	−0.16 (−0.20, −0.11)	**1.07×10^{-13}**	0.13 (0.09, 0.17)	**3.95×10^{-10}**	0.95 (0.89, 1.02)	0.138
Leptin	−0.30 (−0.34, −0.25)	**1.84×10^{-35}**	0.27 (0.22, 0.31)	**7.94×10^{-29}**	0.95 (0.87, 1.03)	0.245
Visfatin	−0.06 (−0.10, −0.03)	**1.04×10^{-3}**	0.07 (0.03, 0.11)	**5.28×10^{-4}**	1.01 (0.95, 1.08)	0.610
PAI-1	−0.13 (−0.17, −0.09)	**1.74×10^{-10}**	0.12 (0.08, 0.16)	**1.39×10^{-9}**	1.05 (0.99, 1.12)	0.089
GIP	−0.16 (−0.20, −0.12)	**2.27×10^{-16}**	0.13 (0.10, 0.17)	**6.61×10^{-12}**	1.56 (1.46, 1.67)	**6.37×10^{-38}**
GLP-1	−0.02 (−0.06, 0.02)	0.421	0.03 (−0.01, 0.07)	0.140	1.07 (1.01, 1.14)	0.034
Ghrelin	−0.08 (−0.12, −0.04)	**5.25×10^{-5}**	0.07 (0.02, 0.10)	**2.11×10^{-3}**	1.02 (0.96, 1.09)	0.471
Resistin	0.07 (0.03, 0.11)	**2.71×10^{-4}**	−0.08 (−0.12, −0.04)	**3.23×10^{-5}**	1.03 (0.97, 1.10)	0.298
IL-6	−0.04 (−0.10, 0.021)	0.226	0.03 (−0.04, 0.09)	0.430	0.91 (0.80, 1.02)	0.115
IL-10	0.11 (0.05, 0.17)	**4.26×10^{-4}**	−0.08 (−0.15, −0.02)	0.011	0.82 (0.70, 0.97)	0.019
IL-1RA	−0.19 (−0.26, −0.13)	**1.06×10^{-8}**	0.15 (0.09, 0.22)	**4.92×10^{-6}**	1.34 (1.17, 1.52)	**1.03×10^{-5}**

Models are adjusted for age, sex, BMI, and recruitment country. HOMA analyses were performed in non-T2D individuals only. Bold indicates significance at a Bonferroni-corrected *P*-value of 0.0045 (= 0.05/11 cytokines/hormones)

Burgess et al. [38]. Given that eight SNPs were associated with other cytokines and hormones in addition to the cytokine/hormone they were selected as instruments for (Additional file 1; Table S1), we were also able to perform multivariable MR analyses in addition to the univariable MR analyses. Using the selected instruments, power to detect an association in MR analyses was higher for the binary outcome T2D (ranging from 0.34 to 1.00) than for the HOMA measures which were analysed in non-T2D individuals only (power ranging from 0.23 to 0.99) (Table 3).

Univariable Mendelian randomization analyses

We found evidence for a causal effect of GIP and resistin on both HOMA-S and HOMA-B in the same direction as in the (traditional) association analysis using univariable

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Table 3 Genetic instruments for all cytokines and hormones at a 5×10^{-7} *p*-value threshold

Cytokine/hormone	Number of participants	Number of genetic instruments	Cumulative F-statistic	Variance explained (R^2)	Power HOMA-S[a]	Power HOMA-B[a]	Power T2D[a]
Adipsin	4854	3	74.8	0.090	0.89	0.88	0.99
Leptin	4914	2	13.6	0.011	0.23	0.23	0.34
Visfatin	4853	2	19.9	0.017	0.28	0.28	0.49
PAI-1	4914	3	20.9	0.026	0.39	0.38	0.66
GIP	4956	7	15.4	0.042	0.57	0.58	0.86
GLP-1	4913	3	15.3	0.018	0.28	0.27	0.51
Ghrelin	4923	2	30.5	0.025	0.38	0.38	0.65
Resistin	4894	7	62.4	0.159	0.99	0.99	1.00
IL-6	1330	1	24.3	0.032	0.46	0.45	0.75
IL-10	1095	2	24.0	0.060	0.74	0.74	0.95
IL-1RA	1416	3	20.5	0.069	0.80	0.79	0.97

[a] Power to detect a β of 0.2 or an OR of 1.5 at an α of 0.05
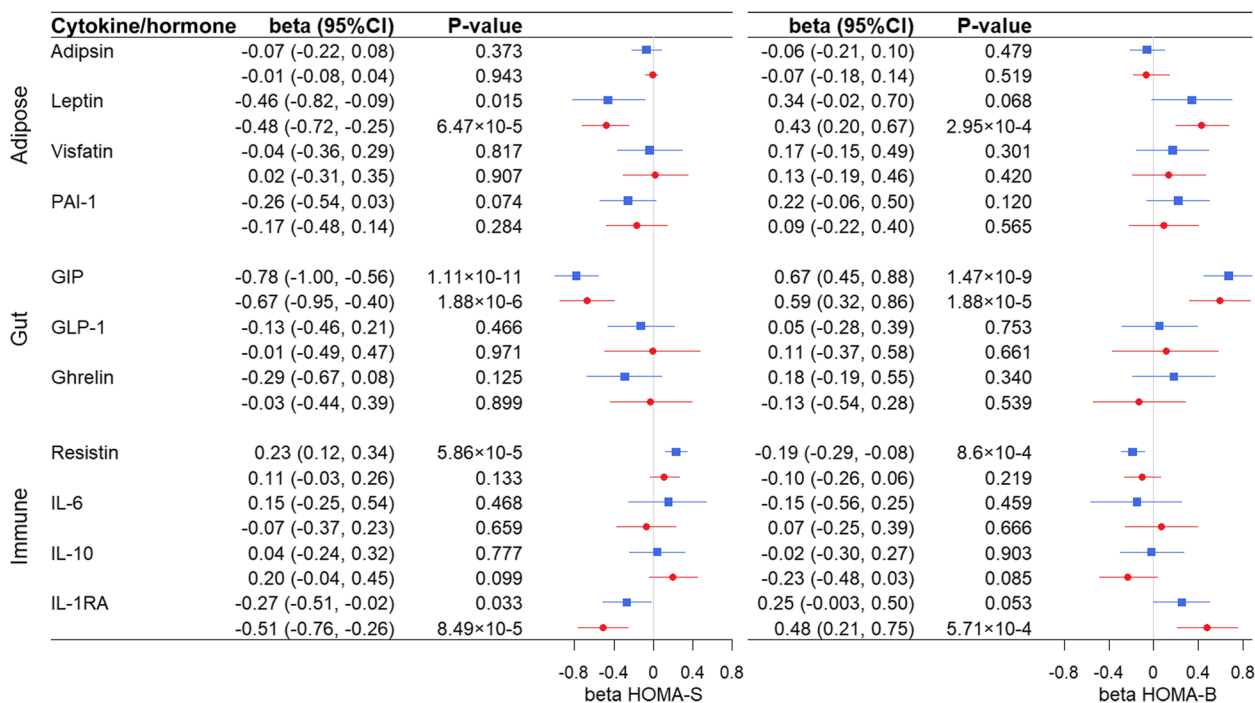

Fig. 3 Forest plot of univariable (blue) and multivariable (red) MR causal associations of cytokines and hormones on HOMA-S (left) and HOMA-B (right). HOMA analyses were performed in non-T2D individuals only. A Bonferroni-corrected *P*-value of < 0.0045 (= 0.05/11 cytokines and hormones) was considered statistically significant

2SLS analyses (Figs. 2B and 3). Genetically predicted higher levels of GIP were associated with lower HOMA-S and with higher HOMA-B, while higher levels of resistin were associated with higher HOMA-S and lower HOMA-B (Fig. 3).

Our univariable MR results using the IVW fixed effects model with T2D as outcome suggested a causal effect of adipsin on T2D (Table 4). While the MR estimate using the median-based method was indicative of a causal effect as well, the association lost statistical significance when using the IVW random effects model and the MR-Egger method (Table 4, Fig. 2B). The I^2 statistic (90.8%) and Cochran's *Q* (21.8) for the fixed effects IVW adipsin model were suggestive of variation across genetic

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Table 4 Univariable MR causal associations of cytokines and hormones on T2D

Cytokine/hormone	IVW Fixed effects		IVW Random effects		Heterogeneity statistics	
	OR (95% CI)	*P*-value	OR (95% CI)	*P*-value	IVW Cochran's Q (*P*-value)	IVW *I*²
Adipsin	**1.74 (1.44, 2.11)**	**8.46 × 10⁻⁹**	1.74 (0.93, 3.26)	0.081	21.81 (< 0.001)	90.8
Leptin	1.20 (0.73, 1.97)	0.470	1.20 (0.73, 1.97)	0.470	0.95 (0.330)	0.0
Visfatin	1.33 (0.87, 2.03)	0.181	1.33 (0.87, 2.03)	0.181	0.92 (0.338)	0.0
PAI-1	0.77 (0.53, 1.13)	0.185	0.77 (0.53, 1.13)	0.185	0.62 (0.735)	0.0
GIP	1.24 (0.96, 1.60)	0.104	1.24 (0.96, 1.60)	0.104	2.54 (0.864)	0.0
GLP-1	0.83 (0.54, 1.27)	0.391	0.83 (0.52, 1.34)	0.444	2.51 (0.285)	20.3
Ghrelin	1.09 (0.77, 1.56)	0.624	1.09 (0.65, 1.84)	0.738	2.15 (0.143)	53.5
Resistin	1.02 (0.89, 1.16)	0.821	1.02 (0.89, 1.16)	0.823	6.13 (0.409)	2.1
IL-6	1.19 (0.86, 1.65)	0.288	1.19 (0.86, 1.65)	0.288	NA	NA
IL-10	1.01 (0.82, 1.25)	0.903	1.01 (0.75, 1.37)	0.931	1.98 (0.159)	49.5
IL-1RA	1.00 (0.81, 1.25)	0.965	1.00 (0.63, 1.61)	0.984	9.18 (0.010)	78.2
	Median		MR-Egger		Heterogeneity statistics	
	OR (95% CI)	*P*-value	OR (95% CI)	*P*-value	MR-Egger Intercept (*P*-value)	MR-Egger *I*²
Adipsin	**1.74 (1.40, 2.17)**	**6.81 × 10⁻⁷**	3.44 (0.81, 14.53)	0.093	−0.225 (0.306)	97.0
Leptin	NA		NA			
Visfatin	NA		NA			
PAI-1	0.76 (0.48, 1.20)	0.237	0.41 (0.08, 2.06)	0.281	0.193 (0.433)	0.0
GIP	1.37 (0.98, 1.90)	0.062	1.41 (0.92, 2.14)	0.111	−0.040 (0.446)	0.0
GLP-1	1.04 (0.59, 1.81)	0.894	0.30 (0.07, 1.29)	0.107	0.226 (0.155)	0.0
Ghrelin	NA		NA			
Resistin	1.03 (0.88, 1.20)	0.709	1.27 (0.93, 1.73)	0.131	−0.108 (0.118)	95.2
IL-6	NA		NA			
IL-10	NA		NA			
IL-1RA	1.08 (0.79, 1.49)	0.621	2.52 (1.24, 5.12)	0.010	−0.384 (0.007)	0.0

The MR-Egger and MR Median analyses could only be performed for cytokines with > 2 genetic instruments. Bold indicates significance at a Bonferroni-corrected *P*-value of < 0.0045 (= 0.05/11 cytokines/hormones)

instruments (Table 4), which can also be seen in Additional file 1; Figure S1 with variant rs80117394 driving the association. We found two of the three genetic instruments for adipsin (rs80117394 and rs854781) associated with BMI at a nominal *P*-value of < 0.01. Hence, we performed an additional analysis excluding these variants as instruments. The IVW estimate for these conservative MR analyses was not statistically significant (OR = 0.61; 95%CI = 0.29, 1.25; *P*-value = 0.175). No evidence for a causal effect of any of the other cytokines or hormones on T2D was found (Table 4). The *I*² statistics and Cochran's *Q* were not indicative of heterogeneity for the other cytokines and hormones, except for IL-1RA (Table 4). For IL-1RA, we also observed a significant MR-Egger intercept, suggesting presence of pleiotropy.

Multivariable Mendelian randomization analyses
The causal effect of GIP on HOMA-S and HOMA-B that we observed in the univariable analyses was independent of other gut-produced hormones (namely, GLP-1 and ghrelin) and had a regression coefficient of − 0.67 for HOMA-S and 0.59 for HOMA-B (Figs. 2C and 3). In comparison with the univariable MR results, the multivariable estimate for resistin on both HOMA-S and HOMA-S was not statistically significant, suggesting that this association was not independent of other cytokines and hormones produced by immune cells. On the other hand, compared to the univariable MR findings, both leptin and IL-1RA gained statistical significance in the multivariable analyses for HOMA-S and HOMA-B (Figs. 2C and 3), suggesting that this cytokine and hormone may have a causal effect on insulin sensitivity and β-cell function independent of other cytokines and hormones. We performed a sensitivity analysis for adipose-produced cytokines and hormones by excluding the genetic instruments that were suggestive of potential pleiotropy (rs80117394, rs854781rs146197730). In this conservative approach, the multivariable MR estimates of leptin on HOMA-S (*β* = − 0.50; 95%CI − 0.91, − 0.09; *P*-value = 0.016) and HOMA-B (*β* = 0.41; 95%CI − 0.01, 0.82; *P*-value = 0.045) lost Bonferroni-corrected statistical significance. Given

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the substantial role of adiposity in the development of glycaemic dysfunction and insulin resistance, we performed mediation analyses to explore whether the causal effect of GIP and IL-1RA may be partially mediated through BMI. We did not find evidence for BMI mediating the effect of GIP on HOMA-S (5.1%, 95%CI = − 0.02, 0.12; *P*-value = 0.17), but BMI did mediate 30.8% of the effect of IL-1RA on HOMA-S (95%CI = 0.20, 0.45; *P*-value < 2×10^{-16}). Similarly, BMI was not a mediator in the effect of GIP on HOMA-B (5.3%; 95%CI = − 0.02, 0.13; *P*-value = 0.18), but it was a mediator in the effect of IL1-RA on HOMA-B (34%; 95%CI = 0.21, 0.53; *P*-value < 2×10^{-16}).

The multivariable MR analyses for T2D showed that the statistically significant estimate of adipsin on T2D with the fixed effect IVW and median methods was independent of other cytokines and hormones produced by adipose tissue (Table 5). However, as with the univariable analyses, the IVW random effects and MR-Egger estimates were not significant, suggesting that the associations in the IVW fixed effects and median methods may be due to pleiotropy. In contrast to the univariable analyses, the leptin IVW fixed effects estimate was statistically significant in the multivariable analyses (Table 5). However, the lack of association in the sensitivity analyses suggests that this estimate is not robust. In addition, one of the two leptin genetic instruments (rs146197730) was associated with sex at a *P*-value of 0.006. The multivariable IVW estimate when excluding this potentially pleiotropic instrument was not statistically significant (*P*-value = 0.226). None of the other cytokines or hormones showed evidence of a causal effect on T2D independent of other cytokines and hormones.

Discussion

We examined the role of 11 circulating cytokines and hormones previously implicated in T2D in predominantly non-human studies. Circulating levels of nine out of the 11 cytokines and hormones were associated with insulin sensitivity and eight with β-cell function among non-T2D individuals. In addition, GIP and IL-1RA were associated with T2D. Furthermore, MR analyses provided evidence for an independent causal effect of circulating GIP levels on insulin sensitivity and β-cell function that was not mediated through BMI and an independent causal effect of IL-1RA on insulin sensitivity and β-cell function that was partially mediated through BMI. For all other cytokines and hormones, no robust evidence for a causal association with insulin sensitivity, β-cell function, or T2D was found.

The plausibility of a causal effect of fasting circulating GIP on reducing insulin sensitivity and promoting β-cell function is supported by evidence from other studies [39, 40]. GIP is an incretin secreted post-prandially by enteroendocrine K-cells found in the gastrointestinal tract, stomach, and pancreas [41]. GIP stimulates the release of insulin from pancreatic β-cells, which facilitates the storage and clearance of dietary triglycerides as well as adipose tissue expansion [41]. High-fat diets in mice were found to induce hypersecretion of GIP [42], and these increased GIP levels have been proposed to play an important role in the reduced insulin sensitivity that is observed in the presence of high-fat-diet consumption and elevated BMI [40, 41]. Despite GIP's suggested role in insulin sensitivity in healthy individuals, GIP resistance has been observed in a T2D state when hyperglycaemia reduces GIP receptor expression in β-cells [43, 44]. In a prospective study of GIP and T2D incidence, fasting

Table 5 Multivariable MR causal associations of cytokines and hormones on T2D

Group	Cytokine/hormone	IVW Fixed effects		IVW Random effects		MR Egger		Median	
		OR (95% CI)	*P*-value	OR (95% CI)	*P*-value	OR (95% CI)	*P*-value	OR (95% CI)	*P*-value
Adipose	Adipsin	**1.83 (1.40, 2.39)**	**9.79×10^{-6}**	1.83 (1.12, 3.00)	0.017	1.67 (0.56, 4.96)	0.357	**2.05 (1.27, 3.31)**	**0.003**
	Leptin	**0.56 (0.40, 0.78)**	**6.33×10^{-4}**	0.56 (0.30, 1.04)	0.065	0.52 (0.18, 1.50)	0.226	0.39 (0.19, 0.81)	0.012
	Visfatin	1.01 (0.66, 1.53)	0.968	1.01 (0.46, 2.19)	0.983	0.96 (0.35, 2.61)	0.932	1.32 (0.53, 3.26)	0.546
	PAI-1	0.94 (0.62, 1.42)	0.760	0.94 (0.43, 2.03)	0.869	0.95 (0.40, 2.25)	0.909	0.89 (0.39, 2.04)	0.776
Gut	GIP	1.37 (1.04, 1.82)	0.026	1.37 (1.04, 1.82)	0.026	1.43 (0.97, 2.10)	0.070	1.41 (0.90, 2.21)	0.139
	GLP-1	0.88 (0.50, 1.54)	0.655	0.88 (0.50, 1.54)	0.655	0.89 (0.51, 1.56)	0.684	1.05 (0.49, 2.25)	0.908
	Ghrelin	0.97 (0.66, 1.42)	0.865	0.97 (0.66, 1.43)	0.865	0.97 (0.66, 1.42)	0.860	0.83 (0.47, 1.48)	0.527
Immune	Resistin	1.03 (0.89, 1.19)	0.724	1.03 (0.84, 1.26)	0.802	1.22 (1.00, 1.49)	0.047	1.03 (0.82, 1.30)	0.776
	IL-6	1.13 (0.84, 1.51)	0.423	1.13 (0.75, 1.70)	0.569	0.97 (0.70, 1.35)	0.868	1.33 (0.89, 1.99)	0.169
	IL-10	0.99 (0.80, 1.24)	0.953	0.99 (0.73, 1.36)	0.966	0.97 (0.77, 1.23)	0.824	1.11 (0.75, 1.64)	0.612
	IL-1RA	0.98 (0.78, 1.22)	0.826	0.98 (0.71, 1.33)	0.875	1.06 (0.83, 1.35)	0.642	0.59 (0.35, 0.99)	0.046

Bold indicates significance at a Bonferroni-corrected *P*-value of < 0.0045 (=0.05/11 cytokines/hormones)

GIP levels were found elevated among normal glycaemic control individuals that developed T2D later [45]. Consistent with these prior observations, we found evidence for a causal effect on insulin sensitivity and β-cell function among non-T2D individuals, but no causal effect on T2D status. While GIP was not previously considered an attractive drug target, recent studies have started re-evaluating GIP's therapeutic potential and have proposed GIP receptor signalling and dual GIP/GLP-1 receptor agonists as a novel means to reduce insulin resistance among T2D cases who have likely developed GIP resistance [46–48].

The role of GIP in insulin secretion is closely linked with GLP-1 but we found no evidence of a causal effect of circulating GLP-1 levels on insulin sensitivity, β-cell function, or T2D. In fact, neither circulating nor genetically predicted GLP-1 was significantly associated with any of these traits. This result may be a consequence of analysing fasting measures in this study as GLP-1 has a more potent action on postprandial insulin secretion in healthy individuals [44]. In addition, we note that a systematic review and meta-analyses of 22 trials found no difference in GLP-1 response between individuals with and without T2D [49]. Hence, our findings are consistent with the possibility that GLP-1 action is affected in T2D rather than GLP-1 circulating levels [49, 50]. Indeed, current GLP-1-based therapies for T2D are based on the activation of GLP-1 receptors through GLP-1 receptor agonists. GLP-1 has a short half-life and is degraded by the dipeptidyl peptidase 4 (DPP-4) enzyme. DPP-4 inhibitors are used in these GLP-1-based therapies to prevent degradation and inactivation of GLP-1 and prolong its action in improving glucose metabolism [47, 51].

The effect of IL-1RA on insulin sensitivity and β-cell function that we found in our study population of sub-Saharan Africans is in line with findings from some studies but in contrast with others. While MR analyses by Nowak et al. were in the same direction of effect as our findings, they did not find evidence for a causal association between IL-1RA and insulin sensitivity [52]. Neither did the Interleukin 1 Genetics Consortium [17] find associations of genetically elevated IL-1RA with insulin sensitivity or T2D. Both studies used data from predominantly European-ancestry populations. On the other hand, a SNP in the *P2RX7* gene was found to be associated with an increase in IL-1RA levels among T2D patients [53], which is consistent with our findings. Findings from randomized controlled trials that studied the effect of the drug Anakinra, which is a recombinant of the naturally occurring IL-1RA and binds to IL-1 receptors, also reported conflicting results with some finding IL-1RA to improve β-cell function [54], whereas others reporting no difference in insulin sensitivity after Anakinra treatment

in obese non-T2D individuals [55]. In our analyses, we were only able to detect the causal association with insulin sensitivity and β-cell function in multivariable MR analyses that adjusted for confounding variables including other cytokines and hormones produced by immune cells, indicating the interplay between these cytokines and hormones and their potential to mask independent effects. Larger studies are needed to optimize IL-1RA genetic instruments for MR studies in diverse populations allowing for better understanding of whether IL-1RA plays a causal role in T2D and related traits.

If our findings of a causal effect of GIP and IL-1RA on insulin sensitivity and β-cell function are confirmed by other studies, there are potential implications for prevention of T2D development. Monitoring of circulating levels could serve as biomarkers for early detection and risk assessment as elevated levels of GIP and IL-1RA could signal increased risk for reduced insulin sensitivity and increased β-cell function. Increased β-cell function often precedes β-cell failure in T2D development [56]. In addition, it could drive further research in the development of novel therapies that target GIP and IL-1RA pathways [57]. There is a need for studies identifying modifiable lifestyle factors driving variation in GIP and IL-1RA levels so that these lifestyle factors can be targeted in intervention strategies among those at elevated risk for T2D.

Our null findings for other circulating cytokines and hormones are consistent with other MR studies that have investigated some of these cytokines and hormones in other populations. Wang et al. did not find evidence for a causal effect of leptin levels on T2D or HOMA measures using data from European-ancestry participants [16] and Song et al. found no evidence for causality of PAI-1 in T2D using SNPs in the *SERPINE1* gene as instrumental variables [58]. The causal effect of resistin levels in insulin sensitivity that our group and others reported previously [59, 60] was found to be dependent of other cytokines and hormones in our present multivariable MR analyses, which is consistent with other studies that evaluated *RETN* SNPs and did not find evidence for an effect of these SNPs on insulin sensitivity [61]. More broadly, Thériault et al. found only six out of 227 studied circulating proteins to be causally associated with blood pressure in a large MR study [62], suggesting most circulating proteins do not have causal roles in cardiometabolic pathologies.

Limitations of our analytic strategy are worth noting. Firstly, we employed a one-sample approach for the MR analyses with the HOMA measures as outcomes, which can lead to increased false positive findings in the presence of weak instruments. We attempted to mitigate this possibility by assessing F-statistics and excluding potentially problematic instruments. We note that no other genetic epidemiological cohort with data on the same

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circulating cytokines and hormones among sub-Saharan Africans exists. This situation presents a challenge considering instrumental variable assumptions are more likely to be violated when two samples represent different ethnic groups [38]. This also applies to the substantial differences in genetic make-up and environmental exposures between sub-Saharan African and African Americans. For adipsin, visfatin, and ghrelin, we are unaware of any other genetic epidemiological cohorts or GWAS summary statistics regardless of ancestry. Secondly, our relatively small sample size resulted in limited power to detect causal relationships for some cytokines and hormones as the power of an MR study increases with its sample size. Thirdly, measurement of the circulating cytokines and hormones in fasting blood samples could have obscured some associations. For example, GIP action differs between a fasting and postprandial state with stronger effects on glucagon and insulin secretion in a postprandial state [63]. Lastly, the generalizability of MR findings across multiple populations warrants careful investigation. There is a need for the inclusion in MR studies of diverse populations with diverse environmental exposures.

Conclusions

In conclusion, this MR study using a sample of sub-Saharan Africans provides evidence for a causal effect of circulating GIP and IL-1RA levels on insulin resistance and β-cell function in non-T2D individuals and suggests that circulating levels of several other cytokines and hormones that have previously been reported in relation to T2D are not causal. While the effect of IL-1RA was partially mediated through BMI, the effect of GIP was not, suggesting that circulating GIP levels could be explored further as a potential biomarker for the development of insulin resistance. Given the few GWAS on circulating cytokines and hormones in general and among sub-Saharan African populations in particular, such studies are needed to expand and further validate these findings in sub-Saharan African and other populations.

Abbreviations

2SLS	Two-stage least squares
AADM	Africa America Diabetes Mellitus Study
BMI	Body mass index
GIP	Glucose-dependent insulinotropic peptide
GLP-1	Glucagon-like peptide-1
GWAS	Genome-wide association study
HOMA-S	Homeostatic Model Assessment of Insulin Sensitivity
HOMA-B	Homeostatic Model Assessment of β-cell Function
IL-10	Interleukin 10
IL-1RA	Interleukin 1 receptor antagonist
IL-6	Interleukin 6
IVW	Inverse variance weighted
MR	Mendelian randomization
ORQ	Ordered quantile
PAI-1	Plasminogen activator inhibitor-1
SNP	Single-nucleotide polymorphism
T2D	Type 2 diabetes

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s13073-023-01263-7.

Additional file 1. Associations of individual genetic instruments with cytokines and hormones. Additional file 1 shows the effect sizes and *P*-values for the associations of all 35 genetic instruments with each of the 11 cytokines and hormones in Table S1. Figure S1 in this file shows the variant-specific estimates as well as the univariable inverse variance weighted (IVW), MR-Egger, and weighted mediation estimates for each of the 11 cytokines and hormones on type 2 diabetes as outcome.

Availability of data and materials
The dataset analysed during the current study is not publicly available due the informed consent obtained which does not grant permission for deposition in an open-access repository of research data. Qualified investigators can gain access to the data as part of a collaboration upon reasonable request consistent with the project's IRB approval and signed informed consent by contacting the Principal Investigator of the AADM study, Dr. Charles Rotimi, at rotimic@mail.nih.gov.

Declarations

Ethics approval and consent to participate
Ethical approval for the AADM study was obtained from the National Institutes of Health (09-HG-N070) and from the respective ethical committees in Ghana (University of Ghana Medical School Research Ethics Committee and the Kwame Nkrumah University of Science and Technology Committee on Human Research Publication and Ethics), Nigeria (National Health Research Ethics Committee of Nigeria [NHREC]), and Kenya (The Moi Teaching & Referral Hospital / Moi University College of Health Sciences -Institutional Research and Ethics Committee [MTRH/MU-IREC]). Informed consent was obtained from all participants prior to enrolment. The research adhered to the declaration of Helsinki.

Consent for publication
Not applicable.

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References

1. Summary Health Statistics. National Health Interview Survey. Selected diseases and conditions among adults aged 18 and over, by selected characteristics: United States. 2018.
2. Meeks KAC, Freitas-Da-Silva D, Adeyemo A, Beune EJ, Modesti PA, Stronks K, et al. Disparities in type 2 diabetes prevalence among ethnic minority groups resident in Europe: a systematic review and meta-analysis. Intern Emerg Med. 2016;11(3):327–40.
3. NCD Risk Factor Collaboration – Africa Working Group. Trends in obesity and diabetes across Africa from 1980 to 2014: an analysis of pooled population-based studies. Int J Epidemiol. 2017;46(5):1421–32.
4. Tsalamandris S, Antonopoulos AS, Oikonomou E, Papamikroulis G-A, Vogiatzi G, Papaioannou S, et al. The role of inflammation in diabetes: current concepts and future perspectives. Eur Cardiol. 2019;14(1):50–9.
5. Wieser V, Moschen AR, Tilg H. Inflammation, cytokines and insulin resistance: a clinical perspective. Arch Immunol Ther Exp. 2013;61(2):119–25.
6. Fu Y, Kaneko K, Lin HY, Mo Q, Xu Y, Suganami T, et al. Gut hormone GIP induces inflammation and insulin resistance in the hypothalamus. Endocrinology. 2020;161(9):bqaa102.
7. Heo YJ, Choi SE, Jeon JY, Han SJ, Kim DJ, Kang Y, et al. Visfatin induces inflammation and insulin resistance via the NF-κB and STAT3 signaling pathways in hepatocytes. J Diabetes Res. 2019;2019:4021623.
8. Lindsay RS, Funahashi T, Hanson RL, Matsuzawa Y, Tanaka S, Tataranni PA, et al. Adiponectin and development of type 2 diabetes in the Pima Indian population. Lancet. 2002;360(9326):57–8.
9. Wang J-S, Lee W-J, Lee I-T, Lin S-Y, Lee W-L, Liang K-W, Sheu WH-H. Association between serum Adipsin levels and insulin resistance in subjects with various degrees of glucose intolerance. J Endocr Soc. 2018;3(2):403–10.
10. Yarmolinsky J, Bordin Barbieri N, Weinmann T, Ziegelmann PK, Duncan BB, Schmidt MI. Plasminogen activator inhibitor-1 and type 2 diabetes: a systematic review and meta-analysis of observational studies. Sci Rep. 2016;6(1):17714.
11. Liu S, Tinker L, Song Y, Rifai N, Bonds DE, Cook NR, et al. A prospective study of inflammatory cytokines and diabetes mellitus in a multiethnic cohort of postmenopausal women. Arch Intern Med. 2007;167(15):1676–85.
12. Charles BA, Doumatey A, Huang H, Zhou J, Chen G, Shriner D, et al. The roles of IL-6, IL-10, and IL-1RA in obesity and insulin resistance in African-Americans. J Clin Endocrinol Metab. 2011;96(12):E2018–22.
13. Meilleur KG, Doumatey A, Huang H, Charles B, Chen G, Zhou J, et al. Circulating adiponectin is associated with obesity and serum lipids in West Africans. J Clin Endocrinol Metab. 2010;95(7):3517–21.
14. Tekola Ayele F, Doumatey A, Huang H, Zhou J, Charles B, Erdos M, et al. Genome-wide associated loci influencing interleukin (IL)-10, IL-1Ra, and IL-6 levels in African Americans. Immunogenetics. 2012;64(5):351–9.
15. Doumatey AP, Lashley KS, Huang H, Zhou J, Chen G, Amoah A, et al. Relationships among obesity, inflammation, and insulin resistance in African Americans and West Africans. Obesity (Silver Spring, Md). 2010;18(3):598–603.
16. Wang X, Jia J, Huang T. Shared genetic architecture and casual relationship between leptin levels and type 2 diabetes: large-scale cross-trait meta-analysis and Mendelian randomization analysis. BMJ Open Diabetes Res Care. 2020;8(1):e001140.
17. The Interleukin 1 Genetics Consortium. Cardiometabolic effects of genetic upregulation of the interleukin 1 receptor antagonist: a Mendelian randomisation analysis. Lancet Diabetes Endocrinol. 2015;3(4):243–53.
18. Schutte AE, Huisman HW, Schutte R, Malan L, van Rooyen JM, Malan NT, Schwarz PE. Differences and similarities regarding adiponectin investigated in African and Caucasian women. Eur J Endocrinol. 2007;157(2):181–8.
19. Degawa-Yamauchi M, Dilts JR, Bovenkerk JE, Saha C, Pratt JH, Considine RV. Lower serum Adiponectin levels in African-American boys. Obes Res. 2003;11(11):1384–90.
20. Carroll JF, Fulda KG, Chiapa AL, Rodriquez M, Phelps DR, Cardarelli KM, et al. Impact of race/ethnicity on the relationship between visceral fat and inflammatory biomarkers. Obesity. 2009;17(7):1420–7.
21. Greyling A, Pieters M, Hoekstra T, Oosthuizen W, Schutte A. Differences in the association of PAI-1 activity with the metabolic syndrome between African and Caucasian women. Nutr Metab Cardiovasc Dis. 2007;17(7):499–507.
22. Rotimi CN, Chen G, Adeyemo AA, Furbert-Harris P, Guass D, Zhou J, et al. A genome-wide search for type 2 diabetes susceptibility genes in West Africans: the Africa America Diabetes Mellitus (AADM) Study. Diabetes. 2004;53(3):838–41.
23. Rotimi CN, Dunston GM, Berg K, Akinsete O, Amoah A, Owusu S, et al. In search of susceptibility genes for type 2 diabetes in West Africa: the design and results of the first phase of the AADM study. Ann Epidemiol. 2001;11(1):51–8.
24. Adeyemo AA, Zaghloul NA, Chen G, Doumatey AP, Leitch CC, Hostelley TL, et al. ZRANB3 is an African-specific type 2 diabetes locus associated with beta-cell mass and insulin response. Nat Commun. 2019;10(1):3195.
25. Faul F, Erdfelder E, Buchner A, Lang A-G. Statistical power analyses using G* Power 3.1: tests for correlation and regression analyses. Behav Res Methods. 2009;41(4):1149–60.
26. R Core Team. R: A language and environment for statistical computing. Austria: R Foundation for Statistical Computing Vienna; 2021. (https://www.R-project.org/).
27. RStudio Team. RStudio: integrated development for R. Boston: RStudio P; 2020. (http://www.rstudio.com/).
28. Peterson RA. Finding optimal normalizing transformations via best normalize. R J. 2021;13(1):294–313.
29. Meeks KAC, Bentley AR, Gouveia MH, Chen G, Zhou J, Lei L, et al. Genome-wide analyses of multiple obesity-related cytokines and hormones informs biology of cardiometabolic traits. Genome Med. 2021;13(1):156.
30. Chang CC, Chow CC, Tellier LC, Vattikuti S, Purcell SM, Lee JJ. Second-generation PLINK: rising to the challenge of larger and richer datasets. GigaScience. 2015;4(1):s13742-015.
31. Brion MJA, Shakhbazov K, Visscher PM. Calculating statistical power in Mendelian randomization studies. Int J Epidemiol. 2012;42(5):1497–501.
32. Kleiber C, & ZeileisA. AER: applied econometrics with R (Version 1.2–5). 2017. Available from: https://CRAN.R-project.org/package=AER.
33. Yavorska OO, Burgess S. MendelianRandomization: an R package for performing Mendelian randomization analyses using summarized data. Int J Epidemiol. 2017;46(6):1734–9.
34. Burgess S, Davies NM, Thompson SG. Bias due to participant overlap in two-sample Mendelian randomization. Genet Epidemiol. 2016;40(7):597–608.
35. Bowden J, Davey Smith G, Burgess S. Mendelian randomization with invalid instruments: effect estimation and bias detection through Egger regression. Int J Epidemiol. 2015;44(2):512–25.
36. Bowden J, Davey Smith G, Haycock PC, Burgess S. Consistent estimation in Mendelian randomization with some invalid instruments using a weighted median estimator. Genet Epidemiol. 2016;40(4):304–14.
37. Tingley D, Yamamoto T, Hirose K, Keele L, Imai K. Mediation: R package for causal mediation analysis. 2014.
38. Burgess S, Davey Smith G, Davies N, Dudbridge F, Gill D, Glymour M, et al. Guidelines for performing Mendelian randomization investigations: update for summer 2023. Wellcome Open Res. 2023;4:186.

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39. Nasteska D, Harada N, Suzuki K, Yamane S, Hamasaki A, Joo E, et al. Chronic reduction of GIP secretion alleviates obesity and insulin resistance under high-fat diet conditions. Diabetes. 2014;63(7):2332–43.

40. Chen S, Okahara F, Osaki N, Shimotoyodome A. Increased GIP signaling induces adipose inflammation via a HIF-1α-dependent pathway and impairs insulin sensitivity in mice. Am J Physiol Endocrinol Metab. 2015;308(5):E414–25.

41. Yamane S, Harada N. Gastric inhibitory polypeptide/glucose-dependent insulinotropic polypeptide signaling in adipose tissue. J Diabetes Investig. 2019;10(1):3–5.

42. Suzuki K, Harada N, Yamane S, Nakamura Y, Sasaki K, Nasteska D, et al. Transcriptional Regulatory Factor X6 (Rfx6) Increases Gastric Inhibitory Polypeptide (GIP) expression in enteroendocrine K-cells and is involved in GIP hypersecretion in high fat diet-induced obesity *. J Biol Chem. 2013;288(3):1929–38.

43. Piteau S, Olver A, Kim S-J, Winter K, Pospisilik JA, Lynn F, et al. Reversal of islet GIP receptor down-regulation and resistance to GIP by reducing hyperglycemia in the Zucker rat. Biochem Biophys Res Commun. 2007;362(4):1007–12.

44. Nauck MA, Quast DR, Wefers J, Pfeiffer AFH. The evolving story of incretins (GIP and GLP-1) in metabolic and cardiovascular disease: a pathophysiological update. Diabetes Obes Metab. 2021;23(S3):5–29.

45. Suh S, Kim MY, Kim SK, Hur KY, Park MK, Kim DK, et al. Glucose-Dependent insulinotropic peptide level is associated with the development of type 2 diabetes mellitus. Endocrinol Metab (Seoul). 2016;31(1):134–41.

46. Irwin N, Gault VA, O'Harte FPM, Flatt PR. Blockade of gastric inhibitory polypeptide (GIP) action as a novel means of countering insulin resistance in the treatment of obesity-diabetes. Peptides. 2020;125:170203.

47. Fisman EZ, Tenenbaum A. The dual glucose-dependent insulinotropic polypeptide (GIP) and glucagon-like peptide-1 (GLP-1) receptor agonist tirzepatide: a novel cardiometabolic therapeutic prospect. Cardiovasc Diabetol. 2021;20(1):225.

48. Samms RJ, Coghlan MP, Sloop KW. How may GIP enhance the therapeutic efficacy of GLP-1? Trends Endocrinol Metab. 2020;31(6):410–21.

49. Calanna S, Christensen M, Holst JJ, Laferrère B, Gluud LL, Vilsbøll T, Knop FK. Secretion of glucagon-like peptide-1 in patients with type 2 diabetes mellitus: systematic review and meta-analyses of clinical studies. Diabetologia. 2013;56(5):965–72.

50. Bagger JI, Knop FK, Lund A, Vestergaard H, Holst JJ, Vilsbøll T. Impaired regulation of the incretin effect in patients with type 2 diabetes. J Clin Endocrinol Metab. 2011;96(3):737–45.

51. Sharma D, Verma S, Vaidya S, Kalia K, Tiwari V. Recent updates on GLP-1 agonists: current advancements & challenges. Biomed Pharmacother. 2018;108:952–62.

52. Nowak C, Sundström J, Gustafsson S, Giedraitis V, Lind L, Ingelsson E, Fall T. Protein biomarkers for insulin resistance and type 2 diabetes risk in two large community cohorts. Diabetes. 2016;65(1):276–84.

53. Uresti-Rivera EE, García-Jacobo RE, Méndez-Cabañas JA, Gaytan-Medina LE, Cortez-Espinosa N, Portales-Pérez DP, et al. The presence of the 1068 G>A variant of P2X7 receptors is associated to an increase in IL-1Ra levels, insulin secretion and pancreatic β-cell function but not with glycemic control in type 2 diabetes patients. Gene. 2018;652:1–6.

54. Larsen CM, Faulenbach M, Vaag A, Vølund A, Ehses JA, Seifert B, et al. Interleukin-1–receptor antagonist in type 2 diabetes mellitus. N Engl J Med. 2007;356(15):1517–26.

55. van Asseldonk EJP, Stienstra R, Koenen TB, Joosten LAB, Netea MG, Tack CJ. Treatment with Anakinra improves disposition index but not insulin sensitivity in nondiabetic subjects with the metabolic syndrome: a randomized, double-blind, placebo-controlled study. J Clin Endocrinol Metab. 2011;96(7):2119–26.

56. Prentki M, Nolan CJ. Islet beta cell failure in type 2 diabetes. J Clin Invest. 2006;116(7):1802–12.

57. Luotola K. IL-1 Receptor Antagonist (IL-1Ra) levels and management of metabolic disorders. Nutrients. 2022;14(16):3422.

58. Song C, Burgess S, Eicher JD, O'Donnell CJ, Johnson AD. Causal effect of plasminogen activator inhibitor type 1 on coronary heart disease. J Am Heart Assoc. 2017;6(6):e004918.

59. Meeks KAC, Doumatey AP, Bentley AR, Gouveia MH, Chen G, Zhou J, et al. Genetics of circulating resistin level, a biomarker for cardiovascular diseases, is informed by Mendelian randomization and the unique characteristics of African genomes. Circ Genom Precis Med. 2020;13(5):488–503.

60. Xu JY, Sham PC, Xu A, Tso AW, Wat NM, Cheng KY, et al. Resistin gene polymorphisms and progression of glycaemia in southern Chinese: a 5-year prospective study. Clin Endocrinol (Oxf). 2007;66(2):211–7.

61. Cho YM, Youn BS, Chung SS, Kim KW, Lee HK, Yu KY, et al. Common genetic polymorphisms in the promoter of resistin gene are major determinants of plasma resistin concentrations in humans. Diabetologia. 2004;47(3):559–65.

62. Thériault S, Sjaarda J, Chong M, Hess S, Gerstein H, Paré G. Identification of circulating proteins associated with blood pressure using Mendelian randomization. Circ Genom Precis Med. 2020;13(1):e002605.

63. El K, Campbell JE. The role of GIP in α-cells and glucagon secretion. Peptides. 2020;125:170213.

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