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Supplementary Materials for

Systematic evaluation of the association between hemoglobin levels and metabolic profile implicates beneficial effects of hypoxia

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Supplementary Text

Supplementary Materials and Methods

Human cohorts

The NFBC1966 originally included 12 068 mothers who gave birth to 12 231 live born children (96.3% of all estimated births in the two northernmost provinces of Finland, Oulu and Lapland, during the year 1966) (11, 12). The data collection started in 1965 when the mothers were pregnant and so far, data has been collected at 1, 14, 31 and 46-years. The YFS includes 4320 boys and girls in six age cohorts (age 3, 6, 9, 12, 15 and 18-years), which were randomly chosen from the national register. The first survey was conducted in 1980 and after that, several follow-up studies of this cohort have been conducted (13).

Blood analyses

Blood samples of NFBC1966 were taken after an overnight fasting period and centrifuged immediately. Before analysing, the samples of 31-years were first stored at −20 °C and later at −80 °C, whereas samples of 46-years were analysed immediately without storing. NFBC1966 blood samples were analysed in NordLab Oulu (former name Oulu University Hospital, Laboratory), a testing laboratory (T113) accredited by Finnish Accreditation Service (FINAS) (EN ISO 15189). For YFS blood analyses, see (13).

Blood Hb level and red blood cell parameters

Blood Hb and red cell parameters of NFBC1966 were determined using a Coulter STKR analyser (Beckman Coulter, Fullerton, CA, USA) at age 31-years and a Sysmex XE-2100 analyser (Sysmex Corporation, Kobe, Japan) at age 46-year. The whole blood Hb levels of NFBC1966 were determined using spectrophotometric methods, red blood cells (Eryt) were measured using electric resistance detecting methods (impedance technology) with hydrodynamic focusing. At age 31-years hematocrit (Hct) was measured by the sum of Eryt counted in a specified volume of diluted blood and at age 46-years Hct were determined applying the Eryt pulse-height detection.

Serum lipids

Serum cholesterol (total, HDL and LDL) and triglycerides of NFBC1966 were determined using a Hitachi 911 automatic analyser and commercial reagents (Roche, Boehringer Mannheim, Germany) at 31-years (47, 48) and an enzymatic assay method (Advia 1800; Siemens Healthcare Diagnostics Inc., Tarrytown, NY, USA) at 46-years.

High sensitivity C-reactive protein (hsCRP)

HsCRP in NFBC1966 was analysed by an immunoenzymometric assay (Medix Biochemica, Espoo, Finland) at 31-years and by an immunonefelometric assay at 46-years (BN ProSpec, Siemens Healthcare Diagnostics Inc., Newark, DE, USA).

Glucose and insulin levels and fasting indices

At age 31-years fasting plasma glucose and serum insulin levels of NFBC1966 were determined by glucose dehydrogenase method (Granutest 250, Diagnostica Merck, Darmstadt, Germany) and by radioimmunoassay (Pharmacia Diagnostics, Uppsala, Sweden), respectively. At 46-years fasting plasma glucose and serum insulin levels of NFBC1966 were analysed by an enzymatic dehydrogenase method (Advia 1800, Siemens Healthcare Diagnostics, Tarrytown, NY, USA) and

by a chemiluminometric immunoassay (Advia Centaur XP, Siemens Healthcare Diagnostics, Tarrytown, NY, USA), respectively. To evaluate the insulin resistance and β-cell function we calculated fasting indices HOMA-IR (fasting plasma glucose x fasting serum insulin / 22.5) and HOMA-β ((20 x fasting serum insulin) / (fasting plasma glucose – 3.5) x 100).

Oral glucose tolerance test (OGTT) and diabetes

A two hour OGTT was performed after overnight (12 h) fasting period for NFBC1966 at 46-years (n = 4446). Medication for diabetes or just before test measured capillary blood glucose level >8.0 mmol/l were used as exclusion criteria. Both serum insulin and plasma glucose were measured at baseline and 30, 60 and 120 min after 75 g glucose intake. OGTT glucose and insulin values were used to calculate insulin and glucose area under curve (AUC) (glucose-AUC and insulin-AUC), Matsuda index for insulin sensitivity (10 000 x ((fasting plasma glucose x fasting serum insulin) x (fasting plasma glucose + 30 min plasma glucose + 60 min plasma glucose + 120 min plasma glucose) / 4) x ((fasting serum insulin + 30 min serum insulin + 60 min serum insulin + 120 min serum insulin) / 4))) (49).

Serum metabolomics

Metabolic measures were obtained using the same high-throughput serum NMR metabolomics platform in the same analysis laboratory as described previously (31). This methodology provides information on 228 serum measures, including lipoprotein subclass distribution and lipoprotein particle concentration, low molecular weight metabolites, such as amino acids, 3-hydroxybutyrate and creatinine, and detailed molecular information on serum lipids. The metabolite data provided by this platform has been utilized in various studies in epidemiology and genetics that have been recently reviewed (50). The method provides metabolic measures in concentration units facilitating interpretation and replication of findings. In this study, we excluded all lipid ratio measures provided by the platform and used 150 metabolites for our analyses.

Anthropometric measurements

Body weight, height and waist and hip circumferences of NFBC1966 participants were measured at 31 and 46-years. Body weight was measured with digital scale, which was calibrated regularly. Height was measured twice (mean of the two measurements was used) by using standard and calibrated stadiometer. Finally, body mass index (BMI) was calculated as the ratio of weight and height squared. Waist and hip circumferences were measured twice and mean of the two measurements was used. The waist-hip ratio was assessed as the ratio between circumferences of the waist (at the level midway between lowest rib margin and the iliac crest) and the hip (at the widest trochanters). In addition, body fat mass, fat percentage, muscle mass and visceral fat area of NFBC1966 participants at 46-years were measured by InBody 720 bioelectrical impedance analyser (Biospace Co., Ltd., Seoul, Korea). All anthropometric measurements were done after overnight (12 h) fasting period. For YFS, see (13).

Brachial blood pressure measurement

In NFBC1966 brachial systolic and diastolic blood pressure was measured two times at 31-years and three times at 46-years with 1 min interval after 15 min of rest on the right arm of the seated participants using an automated oscillometric blood pressure device and appropriately sized cuff (Mercury sphygmomanometer at 31-years and Omron Digital Automatic Blood Pressure Monitor

Model M10-IT at 46-years). Finally, the mean of two lowest systolic values and their diastolic values was used in the analyses.

Confounders and exclusions

Smoking and physical activity were used as confounders. NFBC1966 participants reported their smoking habits at 31- and 46-years. Accordingly, the participants we categorised as never, former and current smokers at both time points. For physical activity, we created a categorical variable based on a question regarding the amount of keep-fit exercise in leisure time. For variable definitions for smoking and physical activity in YFS, see (13). Individuals using combined oral contraceptives, hormone replacement therapy or serum lipids lowering drugs were omitted from the metabolomics analyses, as these have been shown to greatly influence these measures.

Statistical analyses

Oxygen consumption analyses - Regression models of the effects of Hb levels on oxygen consumption at rest divided by height (VO_2rest/h), VO_2rest/h on body fat percentage and Hb levels on body fat percentage were conducted for a subset of NFBC1966 participants at 31-years who underwent a maximal exercise test with direct measurement of oxygen consumption (M901, ergospirometer, Medikro, Finland) (51). These analyses included sex as an additional explanatory variable.

Gene Set Enrichment Analysis (GSEA) - To examine the hypothesized activation of hypoxia response pathway, a GSEA (33) was conducted for a subset of YFS participants at 34-49 years with Hb levels and whole-blood genome-wide expression profiling data (n = 1636) (34). The tested hypoxia response pathway included 22 genes induced at least four-fold by hypoxia in human monocytes (Supplementary Table 10) (52). The GSEA was performed between YFS participants in the lowest (Hb <132, n = 392) and the highest (Hb > 152, n = 371) quartile of Hb distribution. The analysis was adjusted for age, sex, BMI, YFS study center, leukocyte and thrombocyte counts, five first principal components of transcriptomics data and three technical variables from microarray hybridizations (chip, plate and well). The analysis was done with residual gene expression profiles calculated by linear regression of each gene expression probe and above mentioned variables. A nominal P value < 0.05 was considered significant.

NMR metabolomics - In total, 150 metabolites from the NMR metabolomics panel were analyzed (Supplementary Table 9). Of these, 144 were measured in concentrations, and cube-root transformation was applied to these variables (53). The rest of the variables were left untransformed, except the degree of unsaturation, which had a notably skewed distribution and thus log-transformed to ensure homoscedasticity in model residuals. The users of combined oral contraceptives, hormone replacement therapy and lipid lowering drugs (total n = 957) were omitted because their influence on these measures (54, 55). The analyses were conducted first separately in the NFBC1966 (at 46-years) and YFS datasets, and then meta-analyzed using inverse-variance weighted random-effects models. BMI was included as an additional explanatory variable.

Longitudinal models in NFBC1966

Extended linear models using generalized least squares were used to examine the longitudinal change in association between Hb levels and the outcomes available at two time points in the NFBC1966. Time of measurement (31-years or 46-years) was included in the model as a categorical variable, and interaction terms with time and all other variables (excluding sex and height where applicable) were included in the model to allow varying effects between cross-

sectional measurements. The error terms were allowed to be correlated within each individual and heteroskedastic between the time points. These analyses were conducted using the *gls()* function available in the *nlme* package (56). Due to the different methods used to measure hsCRP at 31 and 46 years, an inverse normal rank transformation was applied to set the values on a comparable scale. As the glucose concentrations at the NFBC1966 31-year follow-up were measured from whole blood these values were transformed to corresponding concentrations in plasma (57) for compatibility with the data at 46-year follow-up.

Cross-sectional and longitudinal analysis of Hb levels on metabolites

To analyse both cross-sectional and longitudinal effects of Hb levels on the metabolites, Hb levels were decomposed to mean Hb ($\overline{Hb_j}$) for each individual j and variation of Hb from the individual mean $\left(Hb_{jk} - \overline{Hb_j}\right)$ for each individual j at time point k (1 = 31-years, 2 = 46-years). The following model was fit for each metabolite separately:

$metabolite_{jk} = \beta_0 + \beta_C\overline{Hb_j} + \beta_L\left(Hb_{jk} - \overline{Hb_j}\right) + \beta_T Time_k + \beta_S Sex_j + \beta_Z Z_{jk} + \beta_{TZ} Time_k Z_{jk} + \epsilon_{jk}$. This model allows simultaneous estimation of the cross-sectional (between-subject) effect β_C and the longitudinal (within-subject) effect β_L(18) while controlling for potentially time-varying covariates Z_{jk}, in this case BMI, smoking and physical activity. The error terms ϵ_{jk} were allowed to be correlated within each individual and heteroskedastic between the time points. These analyses were conducted using the *gls()* function available in the *nlme* package (58).

Table S1. Characteristics of the NFBC1966 and YFS study populations. The numbers indicate mean (SD) for continuous variables used non-transformed in the analysis, median (interquartile range) continuous variables that were log-transformed prior to analysis, and number of observations (percentage) for categorical variables. NA = not available.

		NFBC1966 at age of 46		YFS		NFBC1966 at age of 31	
Variable	Units	Males (N = 2315)	Females (N = 3036)	Males (N = 811)	Females (N = 1013)	Males (N = 1551)	Females (N = 2073)
Age	years	46.7 (0.6)	46.7 (0.6)	41.8 (5.1)	41.9 (5.0)		
Hb	g/l	151.2 (9.1)	133.2 (10.6)	151.7 (9.2)	133.1 (9.7)	151.7 (8.7)	132.2 (9.2)
Hct	%	44.3 (2.4)	39.9 (2.7)	44.6 (2.5)	39.9 (2.6)	NA	NA
Eryt	E12/l	4.95 (0.33)	4.46 (0.32)	4.97 (0.33)	4.43 (0.31)	NA	NA
BMI	kg/m^2	26.6 (24.4 - 29.3)	25.3 (22.8 - 29.0)	26.2 (24.0 - 29.2)	25.0 (22.2 - 28.7)	24.9 (22.9 - 26.8)	23.0 (21.1 - 25.7)
Waist circumference	cm	97.3 (11.7)	87.1 (13.0)	96.7 (12.7)	87.5 (13.8)	88.5 (9.3)	78.4 (11.5)
Hip circumference	cm	99.3 (7.8)	100.4 (11.0)	101.2 (7.7)	102.0 (10.0)	97.3 (6.3)	97.0 (8.5)
Height	cm	178.6 (6.2)	164.9 (6.0)	179.9 (6.5)	166.1 (6.0)	178.6 (6.2)	164.9 (6.1)
Weight	kg	87.0 (14.8)	71.9 (14.9)	87.3 (15.8)	71.9 (15.0)	80.0 (11.9)	65.1 (12.7)
Waist-hip ratio		0.98 (0.06)	0.86 (0.06)	0.95 (0.07)	0.85 (0.07)	0.91 (0.06)	0.81 (0.08)
Body fat percentage	%	23.3 (7.1)	33.1 (8.3)	NA	NA	NA	NA
Body muscle mass ratio	%	43.4 (4.1)	36.6 (4.5)	NA	NA	NA	NA
Visceral fat area	cm^2	99.5 (39)	108.5 (42.6)	NA	NA	NA	NA
Fasting insulin	mU/l	8.8 (5.7 - 12.8)	7.3 (5.0 - 10.9)	7.7 (4.8 - 12.1)	6.9 (4.1 - 10.9)	7.7 (6.3 - 9.6)	7.3 (6.0 - 9.1)
Fasting glucose	mmol/l	5.7 (0.8)	5.3 (0.6)	5.6 (0.8)	5.2 (1.0)	5.6 (0.7)	5.3 (0.6)
HOMA-IR		1.2 (0.8 - 1.7)	1.0 (0.7 - 1.4)	1.9 (1.1 - 3.1)	1.6 (0.9 - 2.6)	1.0 (0.8 - 1.3)	0.9 (0.8 - 1.2)
HOMA-B	%	82.5 (64.8 - 103.2)	83.4 (67.1 - 104.3)	NA	NA	91.9 (78.7 - 107.7)	97.8 (84.5 - 114.3)
Triglycerides	mmol/l	1.25 (0.90 - 1.75)	0.92 (0.72 - 1.25)	1.26 (0.95 - 1.87)	0.95 (0.75 - 1.26)	1.08 (0.79 - 1.54)	0.89 (0.67 - 1.25)
AUC of glucose in OGTT	mmol*h/l	7.1 (6.2 - 8.2)	6.3 (5.6 - 7.4)	NA	NA	NA	NA
AUC of insulin in OGTT	mU*h/l	56.4 (38.4 - 91.9)	48.0 (35.1 - 74.4)	NA	NA	NA	NA
2-hour glucose	mmol/l	6.0 (1.8)	5.8 (1.5)	NA	NA	NA	NA
Matsuda index		80.3 (49.0 - 123.0)	100.8 (64.5 - 143.6)	NA	NA	NA	NA
Total cholesterol	mmol/l	5.5 (1.0)	5.2 (0.8)	5.3 (1.0)	5.1 (0.9)	5.2 (1.0)	4.9 (1.0)
HDL cholesterol	mmol/l	1.4 (0.3)	1.7 (0.4)	1.2 (0.3)	1.4 (0.3)	1.4 (0.3)	1.7 (0.4)
LDL cholesterol	mmol/l	3.7 (0.9)	3.2 (0.9)	3.4 (0.9)	3.2 (0.7)	3.2 (0.9)	2.8 (0.8)
Systolic blood pressure	mmHg	128.1 (13.8)	118.1 (14.9)	122.1 (13.0)	114.5 (13.0)	129.6 (12.3)	119.5 (12.0)

Diastolic blood pressure	mmHg	85.6 (10.0)	81.2 (10.3)	76.9 (10.8)	71.7 (9.2)	79.7 (11.1)	74.3 (10.6)
C-reactive protein	mg/l	0.72 (0.40 - 1.44)	0.76 (0.36 - 1.66)	0.79 (0.36 - 1.56)	0.80 (0.34 - 2.14)	0.62 (0.34 - 1.37)	0.85 (0.38 - 2.27)
Antihypertensive medication		74 (3)	30 (1)	80 (10)	96 (9)	30 (2)	34 (2)
Lipid lowering medication		273 (12)	406 (13)	47 (6)	20 (2)	30 (2)	34 (2)
Combined oral contraceptive or hormone replacement therapy		Not on medication	278 (9)	Not on medication	113 (11)	Not on medication	522 (25)
Physical activity	Inactive	680 (29)	745 (25)	NA	NA	522 (34)	672 (32)
	Moderate	1287 (56)	1794 (59)	NA	NA	812 (52)	1164 (56)
	High	348 (15)	497 (16)	NA	NA	217 (14)	237 (11)
Physical activity index		NA	NA	8.9 (1.9)	9.1 (1.9)	NA	NA
Smoking	Current	501 (22)	518 (17)	140 (17)	124 (12)	449 (29)	437 (21)
	Non-smoker			671 (83)	889 (88)		
	Never	1103 (48)	1776 (58)			753 (49)	1213 (59)
	Former	711 (31)	742 (24)			349 (23)	423 (20)

Table S2. Effect sizes for association of Hb levels with BMI and other anthropometric measures. Number of participants (N) in the statistical analyses, effect size estimates (Beta) in units of one standard deviation (1-SD) change in anthropometric measures per 1-SD change in Hb, lower and upper limit for 95% confidence intervals (CIL, CIU) and *P* values for the effect size estimate. NFBC1966 = Northern Finland Birth Cohort 1966; YFS = Young Finns Study; META = meta-analysis of NFBC1966 and YFS using inverse-variance weighted random-effects model. The results are presented for data with extreme values (more than ± 3 SD from the mean) excluded and for full data.

Values with ± 3 SD exclusions						
Parameter	Cohort	N	Beta	CIL	CIU	P
log(BMI)	NFBC1966	5273	0.30	0.26	0.33	4.73e-58
Waist circumference	NFBC1966	5225	0.28	0.24	0.31	2.29e-61
Hip circumference	NFBC1966	5218	0.26	0.23	0.30	3.38e-46
Waist-hip ratio	NFBC1966	5243	0.17	0.14	0.19	3.34e-37
Body fat percentage	NFBC1966	5203	0.24	0.21	0.27	4.47e-55
Visceral fat area	NFBC1966	5167	0.28	0.25	0.32	4.12e-54
Body muscle mass ratio	NFBC1966	5200	-0.20	-0.23	-0.17	2.9e-45
log(BMI)	YFS	1794	0.23	0.17	0.30	5.03e-12
Waist circumference	YFS	1785	0.21	0.14	0.27	7.61e-11
Hip circumference	YFS	1786	0.19	0.13	0.26	6.55e-09
Waist-hip ratio	YFS	1797	0.16	0.10	0.21	1.13e-08
log(BMI)	META	7067	0.27	0.21	0.33	2.17e-19
Waist circumference	META	7010	0.25	0.18	0.31	2.81e-12
Hip circumference	META	7004	0.23	0.17	0.30	1.55e-12
Waist-hip ratio	META	7040	0.17	0.14	0.19	6.34e-45
All values						
Parameter	Cohort	N	Beta	CIL	CIU	P
log(BMI)	NFBC1966	5350	0.28	0.24	0.31	1.46e-54
Waist circumference	NFBC1966	5312	0.26	0.22	0.29	2.63e-54
Hip circumference	NFBC1966	5310	0.24	0.21	0.28	1.37e-42
Waist-hip ratio	NFBC1966	5310	0.16	0.13	0.18	6.87e-33
Body fat percentage	NFBC1966	5258	0.23	0.02	0.26	1.63e-51
Visceral fat area	NFBC1966	5258	0.27	0.23	0.30	2.36e-50
Body muscle mass ratio	NFBC1966	5258	-0.19	-0.22	-0.16	4.69e-42
log(BMI)	YFS	1814	0.19	0.13	0.25	4.07e-09
Waist circumference	YFS	1813	0.16	0.10	0.22	3.31e-07
Hip circumference	YFS	1812	0.14	0.08	0.20	1.53e-05
Waist-hip ratio	YFS	1812	0.12	0.07	0.17	7.26e-06
log(BMI)	META	7164	0.24	0.15	0.32	3.05e-08
Waist circumference	META	7125	0.21	0.11	0.31	2.47e-05
Hip circumference	META	7122	0.19	0.09	0.30	0.000232
Waist-hip ratio	META	7122	0.15	0.11	0.18	1.72e-19

Table S3. Examples of effects of Hb level changes on BMI and other anthropometric measures. The data are from the meta-analysis presented in Supplementary Table S2 (values with ± 3 SD exclusions).

	Unit	Effect by 10-unit change in Hb (95% CI)
BMI	%	3.5 (2.9, 4.1)[a]
Waist circumference	cm	2.5 (1.9, 3.1)
Hip circumference	cm	1.5 (1.0, 2.1)
Waist-hip-ratio	ratio	0.011 (0.0092, 0.012)
Body fat percentage	percentage point	1.7 (1.5, 1.9)
Visceral fat area	cm^2	8.6 (7.5, 9.6)
Body muscle mass ratio	percentage point	-0.85 (-0.96, -0.73)

[a]In an individual with BMI of 25.0 kg/m^2 the increase of 10 g/l in Hb would correspond to an increase to 25.9 kg/m^2.

Table S4. Effect sizes for association of hematocrit levels and red blood cell counts with BMI and other anthropometric measures. Number of participants in the statistical analyses (N), effect sizes (Beta) in units of 1-SD change in anthropometric measures by 1-SD change in hematocrit (Hct) or red blood cell counts (Eryt) and lower and upper limit for 95% confidence intervals (CIL, CIU) of meta-analysis of the NFBC1966 at age of 46 years and YFS at age of 42 years.

	Values with ± 3 SD exclusions					All values				
Hct	N	Beta	CIL	CIU	P	N	Beta	CIL	CIU	P
log(BMI)	7103	0.26	0.21	0.30	2.00e-31	7156	0.26	0.21	0.31	8.90e-23
Waist circumference	7048	0.25	0.22	0.28	9.00e-61	7110	0.25	0.21	0.29	5.40e-42
Hip circumference	7041	0.23	0.16	0.29	3.70e-12	7108	0.23	0.16	0.31	5.80e-10
Waist-hip-ratio	7078	0.15	0.13	0.17	1.30e-42	7120	0.16	0.13	0.18	3.90e-43
Body fat percentage	5227	0.22	0.19	0.25	4.90e-52	5258	0.23	0.20	0.26	5.30e-55
Visceral fat area	5192	0.26	0.23	0.30	8.80e-53	5258	0.27	0.24	0.30	2.60e-57
Body muscle mass ratio	5225	-0.19	-0.21	-0.16	2.10e-42	5258	-0.19	-0.22	-0.16	4.80e-44
	Values with ± 3 SD exclusions					All values				
Eryt	N	Beta	CIL	CIU	P	N	Beta	CIL	CIU	P
log(BMI)	7104	0.34	0.32	0.37	7.40e-126	7157	0.32	0.29	0.34	7.00e-133
Waist circumference	7048	0.31	0.28	0.34	9.60e-123	7110	0.32	0.29	0.34	2.70e-129
Hip circumference	7042	0.31	0.27	0.36	5.00e-41	7109	0.31	0.27	0.36	6.70e-36
Waist-hip-ratio	7080	0.19	0.13	0.26	2.50e-10	7122	0.19	0.13	0.26	3.00e-10
Body fat percentage	5227	0.27	0.24	0.30	4.40e-85	5258	0.28	0.25	0.30	8.70e-89
Visceral fat area	5192	0.32	0.29	0.35	3.70e-86	5258	0.33	0.30	0.36	2.40e-95
Body muscle mass ratio	5225	-0.23	-0.26	-0.20	3.80e-70	5258	-0.23	-0.26	-0.21	5.30e-72

Table S5. Effect sizes for association between Hb levels, oxygen consumption at rest and body fat percentage in a subset of NFBC1966 at age 31. Number of participants (N) in the statistical analyses, effect sizes (Beta) in 1-SD unit changes, lower and upper limit for 95% confidence intervals (CIL, CIU) and *P* values for the effect size. All effects were adjusted for sex.

Effect of	N	Beta	CIL	CIU	P
Hb level on oxygen consumption at rest	114	0.26	0.04	0.48	0.02
Oxygen consumption at rest on body fat percentage	121	0.19	0.08	0.31	0.001
Hb level on body fat percentage	117	0.14	0.00	0.29	0.057

Table S6. Effect sizes for association of Hb levels with glucose and lipid metabolism, blood pressure and inflammatory parameters. Number of participants (N) in the statistical analyses, effect sizes (Beta) in units of 1-SD change in the indicated parameter by 1-SD change in Hb, lower and upper 95% confidence intervals (CIL, CIU) and *P* values for the effect size in NFBC1966 at age of 46. When indicated the data were adjusted for BMI. Users of antihypertensive drugs were excluded from the systolic and diastolic blood pressure analyses.

Values with ± 3 SD exclusions

Parameter	N	Beta	CIL	CIU	P	BMI adjusted
log(fasting insulin)	6393	0.30	0.27	0.33	2.0e-73	no
Fasting glucose	6366	0.16	0.09	0.22	1.0e-06	no
HOMA-IR	6214	0.29	0.26	0.32	1.7e-66	no
HOMA-B	4407	0.20	0.16	0.24	8.8e-24	no
log(AUC of glucose)	4429	0.20	0.16	0.24	1.3e-25	no
log(AUC of insulin)	4452	0.29	0.25	0.33	7.6e-51	no
2 h glucose	4447	0.13	0.09	0.17	1.2e-10	no
log(Matsuda index)	4400	0.33	0.29	0.37	1.8e-64	no
Total cholesterol	7098	0.15	0.12	0.18	8.7e-21	no
HDL cholesterol	7079	-0.17	-0.20	-0.14	5.1e-30	no
LDL cholesterol	7057	0.19	0.13	0.24	1.4e-10	no
Triglycerides	7094	0.26	0.23	0.29	2.2e-67	no
Systolic blood pressure	6111	0.16	0.11	0.21	2.2e-09	no
Diastolic blood pressure	6125	0.28	0.23	0.34	4.4e-22	no
log(C-reactive protein)	7103	0.11	0.03	0.19	0.0079	no
log(fasting insulin)	6382	0.16	0.13	0.19	8.7e-29	yes
Fasting glucose	6355	0.08	0.04	0.12	2.7e-05	yes
HOMA-IR	6203	0.15	0.12	0.18	4.7e-26	yes
HOMA-B	4406	0.10	0.06	0.13	5.1e-07	yes
log(AUC of glucose)	4428	0.11	0.08	0.15	2.4e-09	yes
log(AUC of insulin)	4451	0.17	0.14	0.21	1.7e-21	yes
2 h glucose	4446	0.05	0.01	0.08	0.023	yes
log(Matsuda index)	4399	0.18	0.15	0.22	7.6e-28	yes
Total cholesterol	7089	0.14	0.11	0.17	2.6e-17	yes
HDL cholesterol	7068	-0.09	-0.12	-0.06	1.8e-10	yes
LDL cholesterol	7048	0.16	0.13	0.19	2.0e-24	yes
Triglycerides	7084	0.17	0.14	0.20	2.1e-32	yes
Systolic blood pressure	6105	0.09	0.06	0.12	8.5e-08	yes
Diastolic blood pressure	6119	0.20	0.17	0.23	5.0e-34	yes
log(C-reactive protein)	7092	-0.01	-0.08	0.05	0.72	yes

All values

Parameter	N	Beta	CIL	CIU	P	BMI adjusted
log(fasting insulin)	6466	0.26	0.23	0.29	1.45e-56	no
Fasting glucose	6467	0.09	-0.03	0.21	0.159	no
HOMA-IR	6281	0.25	0.22	0.28	5.18e-51	no
HOMA-B	4457	0.18	0.14	0.22	2.15e-19	no

log(AUC of glucose)	4478	0.19	0.15	0.22	3.65e-22	no
log(AUC of insulin)	4503	0.27	0.23	0.30	9.81e-44	no
2 h glucose	4557	0.13	0.09	0.17	5.71e-11	no
log(Matsuda index)	4450	0.29	0.25	0.32	1.21e-50	no
Total cholesterol	7172	0.15	0.11	0.19	8.71e-14	no
HDL cholesterol	7172	-0.15	-0.18	-0.12	1.22e-24	no
LDL cholesterol	7131	0.19	0.16	0.22	7.11e-34	no
Triglycerides	7174	0.25	0.22	0.28	1.33e-64	no
Systolic blood pressure	6183	0.15	0.10	0.20	3.42e-09	no
Diastolic blood pressure	6183	0.28	0.24	0.32	8.85e-44	no
log(C-reactive protein)	7153	0.10	0.03	0.17	0.00655	no
log(fasting insulin)	6455	0.13	0.09	0.16	1.21e-12	yes
Fasting glucose	6456	0.02	-0.07	0.11	0.635	yes
HOMA-IR	6270	0.12	0.09	0.15	1.01e-16	yes
HOMA-B	4456	0.07	0.04	0.11	6.36e-05	yes
log(AUC of glucose)	4477	0.10	0.06	0.13	1.78e-07	yes
log(AUC of insulin)	4502	0.15	0.11	0.18	3.84e-17	yes
2 h glucose	4556	0.04	0.00	0.08	0.0322	yes
log(Matsuda index)	4449	0.15	0.11	0.18	8.23e-19	yes
Total cholesterol	7161	0.14	0.10	0.17	1.36e-13	yes
HDL cholesterol	7161	-0.08	-0.12	-0.04	0.000236	yes
LDL cholesterol	7121	0.15	0.12	0.19	4.31e-23	yes
Triglycerides	7163	0.17	0.13	0.21	3.15e-14	yes
Systolic blood pressure	6177	0.08	0.05	0.12	1.56e-07	yes
Diastolic blood pressure	6177	0.19	0.16	0.22	1.79e-32	yes
log(C-reactive protein)	7142	-0.02	-0.08	0.05	0.597	yes

Table S7. Comparison of effect sizes for association of Hb levels with BMI and other anthropometric measures at age 31 and 46 in NFBC1966. Age, effect sizes (Beta) in units of 1-SD change in the indicated parameter by 1-SD change in Hb, lower and upper limit for 95% confidence intervals (CIL, CIU) and *P* values for the change in effect size from age 31 to 46 in NFBC1966 cohort.

Values with ± 3 SD exclusions					
Parameter	Age	Beta	CIL	CIU	P
log(BMI)	31	0.18	0.16	0.20	
log(BMI)	46	0.16	0.14	0.19	0.11
Waist circumference	31	0.17	0.15	0.20	
Waist circumference	46	0.20	0.17	0.22	0.013
Hip circumference	31	0.17	0.15	0.19	
Hip circumference	46	0.14	0.12	0.16	0.0039
Waist-hip ratio	31	0.11	0.08	0.13	
Waist-hip ratio	46	0.17	0.14	0.19	3.5e-7
All values					
Parameter	Age	Beta	CIL	CIU	P
log(BMI)	31	0.18	0.15	0.20	
log(BMI)	46	0.16	0.14	0.18	0.12
Waist circumference	31	0.17	0.15	0.19	
Waist circumference	46	0.20	0.17	0.22	0.023
Hip circumference	31	0.19	0.17	0.22	
Hip circumference	46	0.15	0.13	0.18	0.0022
Waist-hip ratio	31	0.09	0.06	0.11	
Waist-hip ratio	46	0.15	0.13	0.17	1.1e-6

Table S8. Comparison of effect sizes for association of Hb levels with glucose and lipid metabolism, blood pressure and inflammatory parameters at age 31 and 46 in NFBC1966. Age, effect sizes (Beta) in units of 1-SD change in the indicated parameter per 1-SD change in Hb, lower and upper limit for 95% confidence intervals (CIL, CIU) and *P* values for the change in effect size from age 31 to 46 of the NFBC1966.

Values with ± 3 SD exclusions

Parameter	Age	Beta	CIL	CIU	P
log(fasting insulin)	31	0.11	0.09	0.13	
log(fasting insulin)	46	0.18	0.15	0.21	2.4e-7
Fasting glucose	31	0.06	0.03	0.08	
Fasting glucose	46	0.08	0.05	0.10	0.12
Triglycerides	31	0.07	0.04	0.10	
Triglycerides	46	0.17	0.15	0.20	6.9e-11
Total cholesterol	31	0.12	0.09	0.15	
Total cholesterol	46	0.18	0.14	0.21	5.6e-4
HDL cholesterol	31	-0.05	-0.07	-0.02	
HDL cholesterol	46	-0.05	-0.08	-0.03	0.53
LDL cholesterol	31	0.13	0.10	0.16	
LDL cholesterol	46	0.20	0.17	0.23	6.0e-6
Systolic blood pressure	31	0.11	0.08	0.13	
Systolic blood pressure	46	0.11	0.08	0.14	0.95
Diastolic blood pressure	31	0.28	0.24	0.31	
Diastolic blood pressure	46	0.20	0.17	0.24	3.6e-5
HOMA-IR	31	0.13	0.10	0.15	
HOMA-IR	46	0.19	0.16	0.22	7.8e-6
HOMA-B	31	0.09	0.06	0.11	
HOMA-B	46	0.13	0.10	0.16	0.0077
C-reactive protein	31	-0.10	-0.13	-0.06	
C-reactive protein	46	0.02	-0.01	0.05	1.7e-12

All values

Parameter	Age	Beta	CIL	CIU	P
log(fasting insulin)	31	0.12	0.09	0.14	
log(fasting insulin)	46	0.17	0.14	0.20	6.8e-4
Fasting glucose	31	0.04	0.01	0.07	
Fasting glucose	46	0.07	0.04	0.11	0.082
Triglycerides	31	0.03	0.00	0.06	
Triglycerides	46	0.13	0.10	0.16	1.3e-11
Total cholesterol	31	0.08	0.05	0.12	
Total cholesterol	46	0.15	0.12	0.18	8.4e-5
HDL cholesterol	31	-0.04	-0.06	-0.01	
HDL cholesterol	46	-0.04	-0.06	-0.01	0.97
LDL cholesterol	31	0.11	0.08	0.14	
LDL cholesterol	46	0.17	0.14	0.20	4.6e-6
Systolic blood pressure	31	0.12	0.09	0.15	

Systolic blood pressure	46	0.11	0.08	0.14	0.61
Diastolic blood pressure	31	0.25	0.22	0.29	
Diastolic blood pressure	46	0.18	0.15	0.21	1.5e-5
HOMA-IR	31	0.11	0.08	0.14	
HOMA-IR	46	0.16	0.12	0.20	0.0083
HOMA-B	31	0.07	0.04	0.11	
HOMA-B	46	0.12	0.08	0.16	0.016
C-reactive protein	31	-0.10	-0.13	-0.06	
C-reactive protein	46	0.02	-0.01	0.04	1.2e-11

Table S9. Effect sizes for association of Hb levels with metabolites. Number of participants (N) in the statistical analyses, effect sizes (Beta) in units of 1-SD change in the indicated parameter by 1-SD change in Hb, lower and upper limit for 95% confidence intervals (CIL, CIU) and *P* values for the effect size of the meta-analysis of NFBC1966 at age of 46 and YFS at age of 42. Results with ± 3 SD exclusions are shown. Users of lipid lowering medication (n = 679) and combined oral contraceptives or hormone replacement therapy (n = 391) were removed from the analyses.

Parameter	N	Beta	CIL	CIU	P
Alanine	6434	0.05	0.01	0.08	0.0058
Glutamine	6413	0.03	0.00	0.07	0.037
Glycine	6392	-0.05	-0.09	-0.01	0.012
Histidine	6402	0.07	0.01	0.14	0.026
Isoleucine	6396	0.12	0.10	0.15	2.6e-19
Leucine	6403	0.20	0.18	0.23	4.8e-54
Valine	6422	0.15	0.12	0.18	2.8e-24
Phenylalanine	6434	0.15	0.11	0.18	8.9e-21
Tyrosine	6412	0.11	0.08	0.14	6.4e-12
Apolipoprotein A-I	6438	-0.04	-0.07	-0.01	0.019
Apolipoprotein B	6429	0.16	0.13	0.20	1.6e-25
Total cholesterol in chylomicrons and extremely large VLDL	6393	0.06	0.01	0.11	0.016
Total cholesterol in very large VLDL	6437	0.10	0.03	0.17	0.0051
Total cholesterol in large VLDL	6398	0.11	0.06	0.17	7.6e-05
Total cholesterol in medium VLDL	6407	0.14	0.11	0.17	6.1e-19
Total cholesterol in small VLDL	6434	0.14	0.10	0.19	1.8e-10
Total cholesterol in very small VLDL	6432	0.07	0.03	0.10	6.8e-05
Total cholesterol in IDL	6437	0.10	0.07	0.13	6.8e-09
Total cholesterol in large LDL	6433	0.12	0.09	0.15	2,00E-12
Total cholesterol in medium LDL	6430	0.14	0.10	0.17	1.4e-15
Total cholesterol in small LDL	6428	0.13	0.10	0.17	2.5e-15
Total cholesterol in very large HDL	6411	-0.08	-0.12	-0.05	5.7e-08
Total cholesterol in large HDL	6398	-0.15	-0.19	-0.12	1.6e-18
Total cholesterol in medium HDL	6442	-0.08	-0.11	-0.04	7.5e-06
Total cholesterol in small HDL	6430	0.14	0.06	0.22	0.00039

Serum total cholesterol	6432	0.10	0.07	0.14	1.9e-09
Total cholesterol in VLDL	6418	0.14	0.11	0.18	1.1e-14
Remnant cholesterol (non-HDL, non-LDL -cholesterol)	6436	0.14	0.11	0.17	1.4e-17
Total cholesterol in LDL	6427	0.13	0.09	0.16	8,00E-14
Total cholesterol in HDL	6436	-0.10	-0.13	-0.07	4.4e-11
Total cholesterol in HDL2	6426	-0.12	-0.15	-0.09	1.4e-15
Total cholesterol in HDL3	6418	0.05	0.02	0.09	0.0024
Esterified cholesterol	6420	0.10	0.06	0.13	1.5e-08
Cholesterol esters in chylomicrons and extremely large VLDL	6399	0.05	-0.02	0.12	0.16
Cholesterol esters in very large VLDL	6437	0.10	0.02	0.18	0.014
Cholesterol esters in large VLDL	6398	0.10	0.04	0.16	0.0014
Cholesterol esters in medium VLDL	6404	0.13	0.08	0.18	4.8e-08
Cholesterol esters in small VLDL	6428	0.13	0.09	0.17	2.1e-09
Cholesterol esters in very small VLDL	6433	0.05	0.02	0.09	0.0015
Cholesterol esters in IDL	6440	0.11	0.07	0.14	4.1e-10
Cholesterol esters in large LDL	6429	0.12	0.09	0.16	1.9e-13
Cholesterol esters in medium LDL	6423	0.13	0.10	0.16	5.9e-15
Cholesterol esters in small LDL	6419	0.13	0.09	0.16	6.2e-14
Cholesterol esters in very large HDL	6407	-0.07	-0.11	-0.04	2.3e-06
Cholesterol esters in large HDL	6398	-0.15	-0.19	-0.12	5.5e-16
Cholesterol esters in medium HDL	6442	-0.08	-0.11	-0.04	4.5e-06
Cholesterol esters in small HDL	6428	0.13	0.06	0.20	0.00012
Total fatty acids	6413	0.13	0.09	0.17	3.9e-11
Estimated degree of unsaturation	6401	-0.08	-0.11	-0.05	2.5e-06
22:6, docosahexaenoic acid	6411	0.07	-0.01	0.14	0.083
18:2, linoleic acid	6409	0.11	0.07	0.14	9.3e-10
Omega-3 fatty acids	6415	0.08	0.05	0.12	1.2e-06
Omega-6 fatty acids	6410	0.10	0.06	0.13	6,00E-08
Polyunsaturated fatty acids	6408	0.10	0.07	0.14	2.7e-09
Monounsaturated fatty acids; 16:1, 18:1	6403	0.16	0.09	0.23	5.00e-06
Saturated fatty acids	6402	0.11	0.06	0.16	7.4e-06
Ratio of monounsaturated fatty acids to total fatty acids	6411	0.15	0.06	0.23	0.001

Creatinine	6425	0.08	0.05	0.11	8.7e-09
Albumin	6413	0.15	0.09	0.21	4.4e-06
Free cholesterol in chylomicrons and extremely large VLDL	6424	0.09	0.06	0.12	5,00E-08
Free cholesterol in very large VLDL	6439	0.10	0.04	0.16	0.0011
Free cholesterol in large VLDL	6438	0.13	0.09	0.17	6.7e-10
Free cholesterol in medium VLDL	6415	0.15	0.13	0.18	3.8e-25
Free cholesterol in small VLDL	6435	0.16	0.13	0.20	1.1e-19
Free cholesterol in very small VLDL	6428	0.09	0.06	0.12	6,00E-08
Free cholesterol in IDL	6424	0.07	0.04	0.11	2.5e-05
Free cholesterol in large LDL	6426	0.09	0.06	0.13	5.2e-08
Free cholesterol in medium LDL	6442	0.15	0.12	0.19	3.5e-19
Free cholesterol in small LDL	6442	0.16	0.13	0.20	6.8e-22
Free cholesterol in very large HDL	6411	-0.11	-0.13	-0.08	2.8e-12
Free cholesterol in large HDL	6398	-0.15	-0.18	-0.12	8,00E-27
Free cholesterol in medium HDL	6433	-0.06	-0.10	-0.03	0.00021
Free cholesterol in small HDL	6444	0.11	0.07	0.15	4.5e-09
Free cholesterol	6422	0.11	0.07	0.14	7.7e-10
Glucose	6384	0.04	-0.08	0.16	0.5
Lactate	6403	0.25	0.21	0.29	9.2e-32
Pyruvate	6425	0.04	0.01	0.08	0.011
Citrate	6427	0.08	-0.00	0.16	0.052
Glycerol	5674	0.20	0.16	0.23	1.1e-29
Glycoprotein acetyls, mainly a1-acid glycoprotein	6411	0.09	0.05	0.13	5.7e-05
Acetate	6411	0.05	-0.01	0.12	0.12
Acetoacetate	6353	0.10	0.07	0.14	3.8e-09
3-hydroxybutyrate	5645	0.12	0.09	0.16	6.1e-11
Mean diameter for VLDL particles	6414	0.13	0.10	0.16	7.3e-17
Mean diameter for LDL particles	6412	-0.14	-0.18	-0.11	2.7e-17
Mean diameter for HDL particles	6437	-0.16	-0.19	-0.14	1.1e-33
Concentration of chylomicrons and extremely large VLDL particles	6384	0.07	0.04	0.10	3.8e-06
Concentration of very large VLDL particles	6441	0.11	0.06	0.16	1.4e-05
Concentration of large VLDL particles	6397	0.12	0.08	0.16	1.4e-09

Concentration of medium VLDL particles	6408	0.15	0.12	0.18	6.9e-25
Concentration of small VLDL particles	6424	0.16	0.13	0.19	4.3e-24
Concentration of very small VLDL particles	6435	0.12	0.09	0.15	2.7e-13
Concentration of IDL particles	6426	0.11	0.07	0.14	6.7e-10
Concentration of large LDL particles	6433	0.12	0.09	0.16	2.9e-13
Concentration of medium LDL particles	6436	0.14	0.11	0.18	2.5e-17
Concentration of small LDL particles	6439	0.15	0.12	0.18	8.6e-19
Concentration of very large HDL particles	6413	-0.11	-0.14	-0.08	1.7e-14
Concentration of large HDL particles	6399	-0.15	-0.18	-0.11	5.7e-16
Concentration of medium HDL particles	6445	-0.05	-0.08	-0.01	0.0051
Concentration of small HDL particles	6446	0.14	0.09	0.20	2.3e-07
Phospholipids in chylomicrons and extremely large VLDL	6423	0.08	0.05	0.11	1.6e-07
Phospholipids in very large VLDL	6440	0.10	0.05	0.16	0.00012
Phospholipids in large VLDL	6397	0.12	0.08	0.16	9.8e-09
Phospholipids in medium VLDL	6408	0.16	0.13	0.18	8.1e-26
Phospholipids in small VLDL	6429	0.16	0.12	0.19	3.8e-19
Phospholipids in very small VLDL	6429	0.10	0.07	0.14	9.6e-10
Phospholipids in IDL	6436	0.09	0.05	0.12	4.8e-07
Phospholipids in large LDL	6436	0.12	0.08	0.15	5.1e-12
Phospholipids in medium LDL	6439	0.16	0.13	0.20	7.4e-23
Phospholipids in small LDL	6442	0.17	0.14	0.20	3.1e-24
Phospholipids in very large HDL	6410	-0.14	-0.17	-0.11	7.8e-23
Phospholipids in large HDL	6399	-0.14	-0.18	-0.11	3.6e-15
Phospholipids in medium HDL	6445	-0.04	-0.08	-0.01	0.0085
Phospholipids in small HDL	6445	0.07	0.03	0.10	0.00012
Total phosphoglycerides	6424	0.08	0.02	0.13	0.0081
Phosphatidylcholine and other cholines	6424	0.04	-0.01	0.10	0.13
Sphingomyelins	6423	0.03	-0.00	0.07	0.082
Total cholines	6428	0.04	0.00	0.07	0.031
Total lipids in chylomicrons and extremely large VLDL	6384	0.07	0.04	0.10	4.5e-06
Total lipids in very large VLDL	6440	0.11	0.06	0.16	3.9e-05
Total lipids in large VLDL	6397	0.12	0.08	0.16	5.2e-09

Total lipids in medium VLDL	6407	0.15	0.12	0.18	6.5e-25
Total lipids in small VLDL	6424	0.16	0.13	0.19	2.5e-23
Total lipids in very small VLDL	6437	0.11	0.08	0.14	9.2e-12
Total lipids in IDL	6425	0.10	0.07	0.14	2.3e-09
Total lipids in large LDL	6432	0.12	0.09	0.16	1,00E-12
Total lipids in medium LDL	6435	0.14	0.11	0.17	3.9e-17
Total lipids in small LDL	6438	0.15	0.11	0.18	1.8e-18
Total lipids in very large HDL	6413	-0.11	-0.14	-0.08	3.6e-14
Total lipids in large HDL	6399	-0.15	-0.18	-0.11	9.6e-17
Total lipids in medium HDL	6445	-0.05	-0.08	-0.02	0.0025
Total lipids in small HDL	6446	0.14	0.08	0.20	1.1e-06
Triglycerides in chylomicrons and extremely large VLDL	6383	0.08	0.04	0.11	1.5e-06
Triglycerides in very large VLDL	6441	0.12	0.07	0.17	1.2e-06
Triglycerides in large VLDL	6398	0.12	0.09	0.16	4.3e-12
Triglycerides in medium VLDL	6414	0.16	0.13	0.19	4.4e-26
Triglycerides in small VLDL	6422	0.16	0.13	0.19	3,00E-28
Triglycerides in very small VLDL	6428	0.15	0.11	0.20	3,00E-12
Triglycerides in IDL	6421	0.11	0.07	0.14	3.3e-10
Triglycerides in large LDL	6418	0.10	0.07	0.13	3.9e-09
Triglycerides in medium LDL	6419	0.10	0.06	0.13	1.4e-08
Triglycerides in small LDL	6420	0.14	0.11	0.17	4,00E-17
Triglycerides in very large HDL	6410	-0.03	-0.06	0.01	0.099
Triglycerides in large HDL	6398	-0.12	-0.15	-0.09	1.4e-13
Triglycerides in medium HDL	6425	0.08	0.03	0.14	0.0043
Triglycerides in small HDL	6429	0.16	0.13	0.19	2.4e-26
Serum total triglycerides	6409	0.16	0.13	0.19	2.7e-25
Triglycerides in VLDL	6415	0.15	0.13	0.18	1.2e-25
Triglycerides in LDL	6417	0.11	0.07	0.14	2.1e-10

Table S10. Hypoxia-induced genes used in the gene set enrichment analysis (GSEA). The genes analysed included those known to be induced at least four-fold by hypoxia in human peripheral blood monocytes (Table modified from (52)). *Genes appearing in multiple functional categories.

Function	Gene Symbol	Gene Description	Fold Change
Angiogenesis	*F3**	Coagulation factor III (tissue factor)	4.5
	FLT1	Vascular endothelial growth factor receptor 1	14.8
	*MIF**	Macrophage migration inhibitory factor	10.1
	*SPP1**	Secreted phosphoprotein 1 (osteopontin)	28.0
	VEGF	Vascular endothelial growth factor	7.5
Apoptosis	*BNIP3*	BCL2/adenovirus E1B 19-kDa-interacting protein 3	16.5
	BNIP3L	BCL2/adenovirus E1B 19-kDa-interacting protein 3-like	4.0
Cell adhesion/matrix/	*F3**	Coagulation factor III (tissue factor)	4.5
	MMP1	Matrix metalloproteinase 1 (interstitial collagenase)	15.6
	*SPP1**	Secreted phosphoprotein 1 (osteopontin)	28.0
Cell cycle/differentation	*INHBA*	Inhibin,β A (activin A)	4.0
	NDRG1	N-myc downstream regulated gene 1	4.1
Glucose transport	*SLC2A3 (GLUT3)*	Solute carrier family 2 (glucose transporter), member 3	5.8
Glycolytic metabolism	*ALDOC*	Aldolase C	5.3
	ENO2	Enolase 2	38.9
	GPI	Glucose phosphate isomerase	7.2
	PDK1	Pyruvate dehydrogenase kinase, isoenzyme 1	24.5
	PFKP	Phosphofructokinase, platelet	7.0
Immune/ responses	*MIF**	Macrophage migration inhibitory factor	10.1
	*SPP1**	Secreted phosphoprotein 1 (osteopontin)	28.0
Metabolism (nonglycolytic)	*EGLN1*	Egl nine homolog 1	4.6
	P4HA1	Procollagen-proline, 2-oxoglutarate 4-dioxygenasa I	4.5
	PAM	Peptidylglycine α-amidating monooxygenase	4.1
	VLDLR	Very low density lipoprotein receptor	5.3
Stress response	*HIG2*	Hypoxia-inducible protein 2	9.7
Transcription/signaling	*MXI1*	MAX-interacting protein 1	4.5

Table S11. Sources for outcome genetic association estimates.

Trait	Population source	Sample size	Population ancestry	Reference
Body mass index (BMI)	GIANT consortium and UK Biobank	806,834	European	(37)
Low-density lipoprotein cholesterol (LDL-C)	GLGC	188,577	European	(38)
High-density lipoprotein cholesterol (HDL-C)	GLGC	188,577	European	(38)
Triglycerides	GLGC	188,577	European	(38)
Systolic blood pressure (SBP)	UK Biobank	318,417	European	(39)
Type 2 diabetes mellitus (T2DM)	DIAMANTE consortium	74,124 cases and 824,006 controls	European	(40)

GIANT: Genetic Investigation of ANthropometric Traits
GLGC: Global Lipids Genetics Consortium
DIAMANTE: DIAbetes Meta-ANalysis of Trans-Ethnic association studies

Table S12. Association of white adipose tissue (WAT) *Slc2a1* mRNA levels with key anthropometric and metabolic markers and Hb levels in a subpopulation of 1-year-old C57Bl/6 male mice (n = 14) (Fig. 7C). *Slc2a1* mRNA levels are expressed relative to *Tbp*.

Mouse	WAT *Slc2a1*	Hb (g/L)	Body weight (g)	2 h glucose of GTT (mmol/L)	WAT weight (g)	Liver weight (g)
1	0.01	17.22	47.40	12.30	2.66	1.87
2	0.01	15.77	41.20	7.60	2.07	2.03
3	0.01	17.43	52.80	11.80	2.81	2.45
4	0.02	17.07	44.90	11.00	2.63	1.99
5	0.02	17.02	48.20	6.00	3.07	1.98
6	0.03	17.13	46.60	9.30	2.85	2.34
7	0.03	16.23	39.40	7.20	1.78	1.88
8	0.04	16.08	39.40	4.90	1.77	1.94
9	0.04	16.83	42.80	7.70	2.04	1.85
10	0.04	16.20	33.70	5.90	0.96	1.62
11	0.04	16.95	37.30	10.90	2.04	1.89
12	0.04	16.00	39.00	6.80	1.50	1.78
13	0.05	15.82	40.20	6.70	1.40	1.74
14	0.06	16.23	34.90	7.40	1.39	1.55
r		-0.51	-0.74	-0.74	-0.69	-0.50
CI 95%		(-0.82; 0.02)	(-0.91; 0.35)	(-0.91; 0.35)	(-0.89; -0.25)	(-0.82; 0.03)
P		0.060	0.002	0.002	0.007	0.066

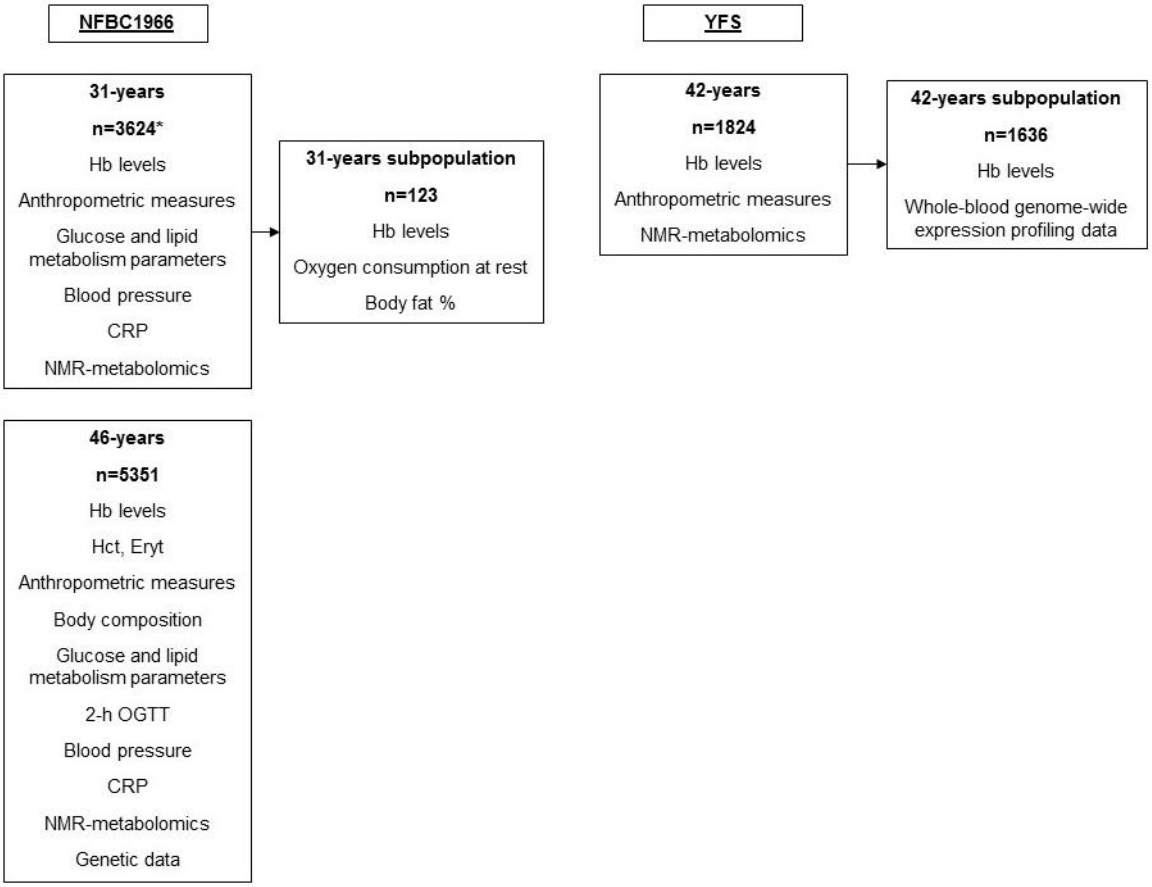

**Fig. S1. Flow chart of the study population. ***Individuals examined for the parameter at the age of 31- and 46-years.

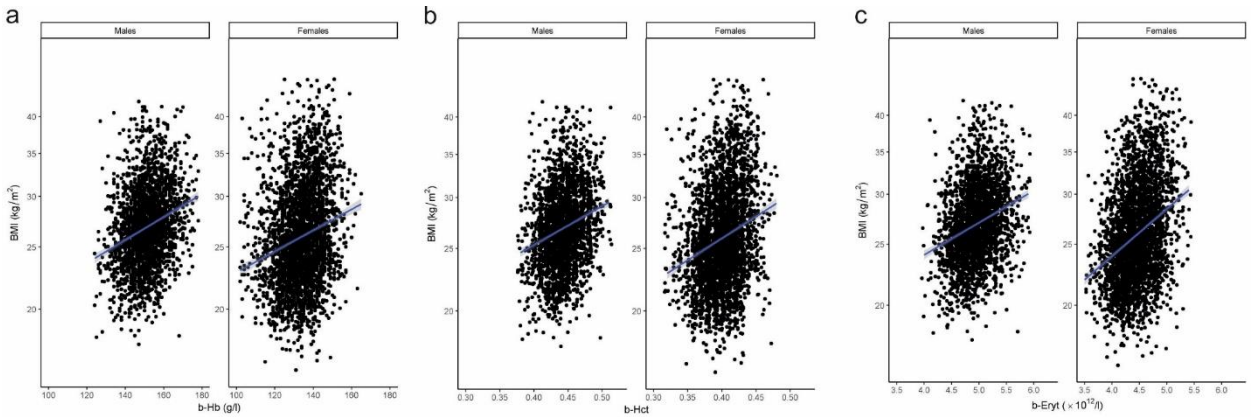

Fig. S2. Red blood cell parameters associate with BMI in both sexes. Scatterplots of (**a**) Hb levels, (**b**) hematocrit (Hct) levels, (**c**) erythrocyte (Eryt) counts, with BMI in both sexes in NFBC1966 at age of 46. Unadjusted regression line (blue) with 95% confidence intervals (gray) are shown.

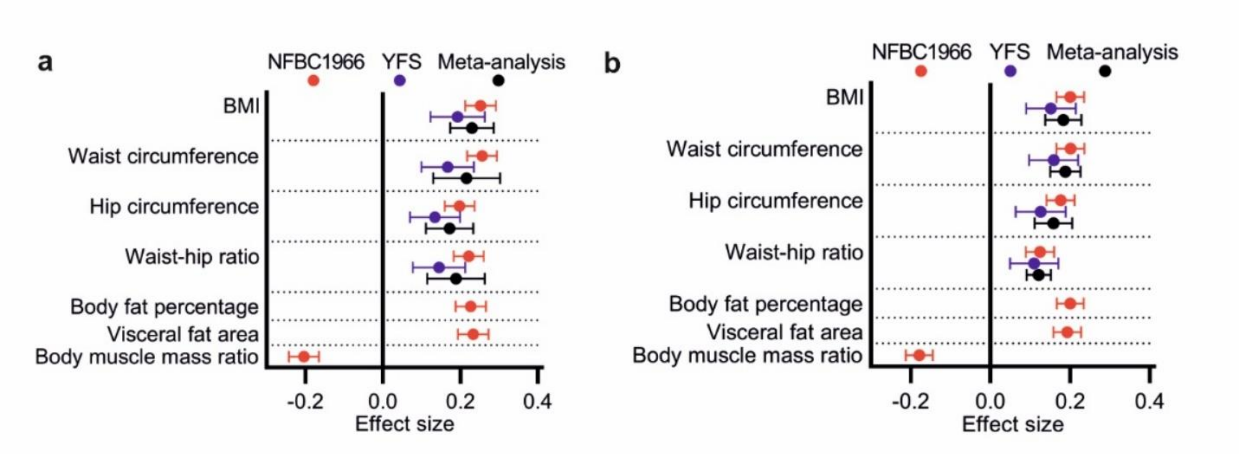

Fig. S3. Association of Hb levels with anthropometric parameters in both sexes. Forest plots representing the effect size estimates and their 95% confidence intervals for one standard deviation (1-SD) change in anthropometric measures per 1-SD change in Hb for both sexes. Red, blue and black lines indicate effect sizes for NFBC1966 at 46-years, YFS at 42-years and meta-analysis, respectively. (**a**) Males, (**b**) females.

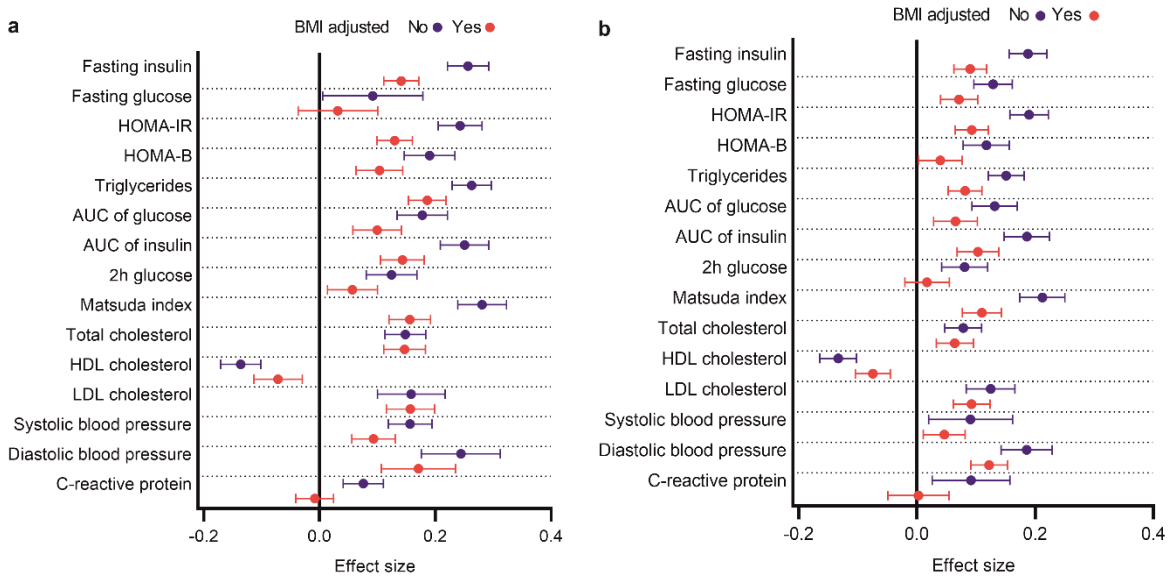

Fig. S4. Association of Hb levels with key metabolic parameters in both sexes. Forest plot representing the effect size estimates and their 95% confidence intervals of the association in SD units of Hb levels with log(fasting insulin) and fasting glucose levels, log(HOMA-IR) and log(HOMA-B) indexes, log(triglycerides), log(area under the curve (AUC) of glucose in oral glucose tolerance test (OGTT))*, log(AUC of insulin in OGTT)* and 2 h glucose in levels in a 2 h OGTT*, log(Matsuda index)*, fasting serum total cholesterol, HDL cholesterol and LDL cholesterol levels, systolic and diastolic blood pressure and log(high-sensitivity C-reactive protein (CRP)) levels in meta-analysis of NFBC1966 at age of 46 and YFS at 42-years, respectively. *Indicates the associations only analyzed in NFBC1966. The effect sizes were adjusted for smoking and physical activity (blue), and additionally for BMI (red). Values with ± 3 SD exclusions are presented. (**a**) Males, (**b**) females.

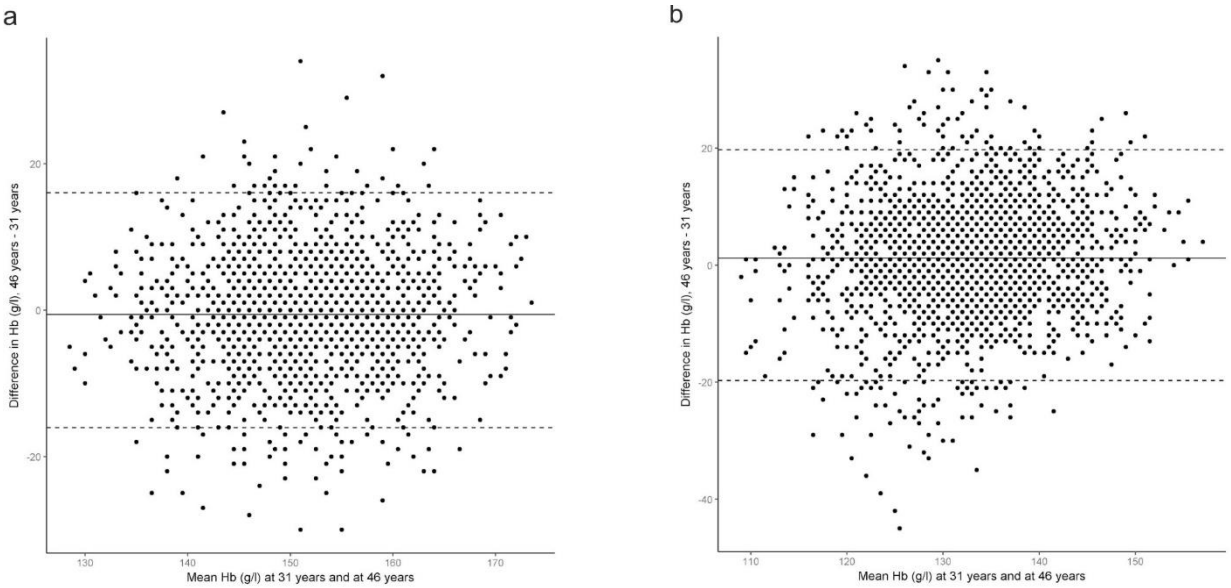

Fig. S5. Bland-Altman plot of Hb levels at age 31 and 46 in both sexes in NFBC1966. (**a**) Males, (**b**) females.

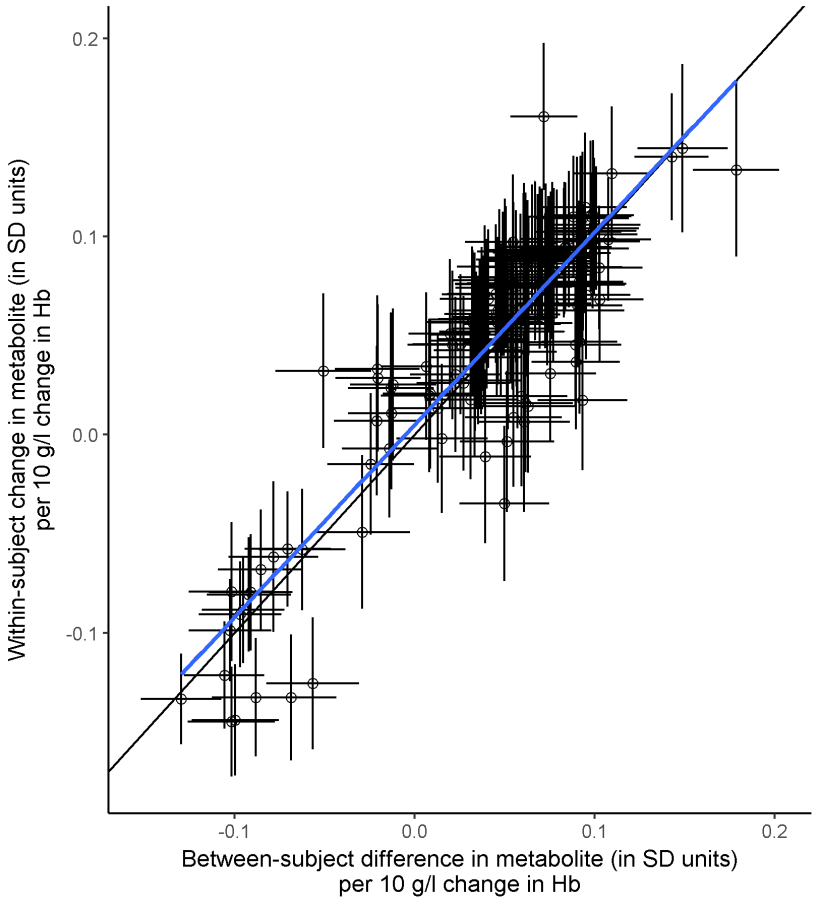

Fig. S6. Effect size estimates and their 95% confidence intervals for cross-sectional and longitudinal associations between Hb levels and metabolites of NFBC1966. X-axis represents between-subject (cross-sectional) effect sizes and y-axis represent within-subject (longitudinal) effect sizes. The solid black line is line y = x indicating equality of the effect sizes. The solid blue line represents the estimated regression line of regressing within-subject effects on between-subject effects.

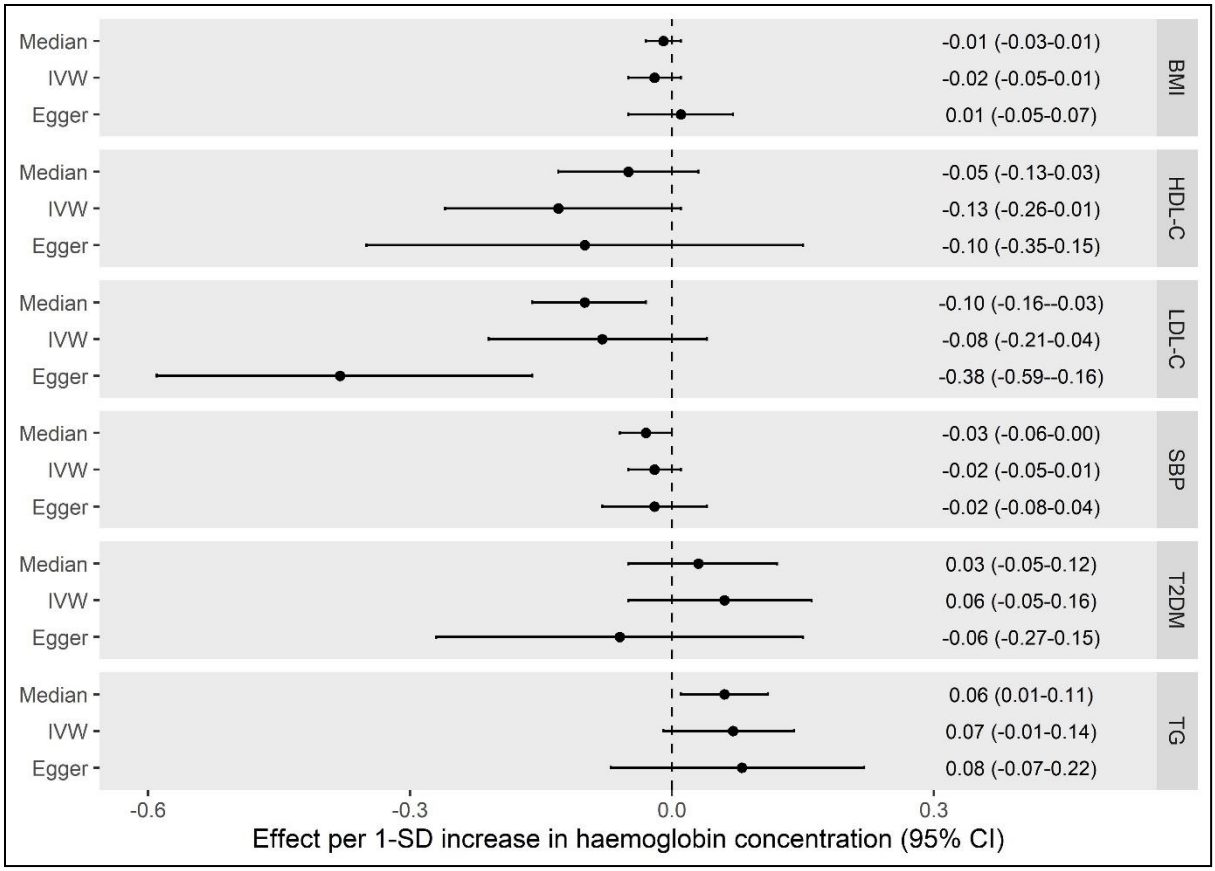

Fig. S7. Main Mendelian randomization analysis results. Effects estimates are given in standard deviation (SD) units for body mass index (BMI), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), systolic blood pressure (SBP) and triglycerides (TG), and in log odds ratio for type 2 diabetes mellitus (T2DM). The Egger intercept *P* values were 0.24 for BMI, 0.80 for HDL-C, 0.002 for LDL-C, 0.98 for SBP, 0.20 for T2DM and 0.92 for TG). IVW: inverse-variance weighted.

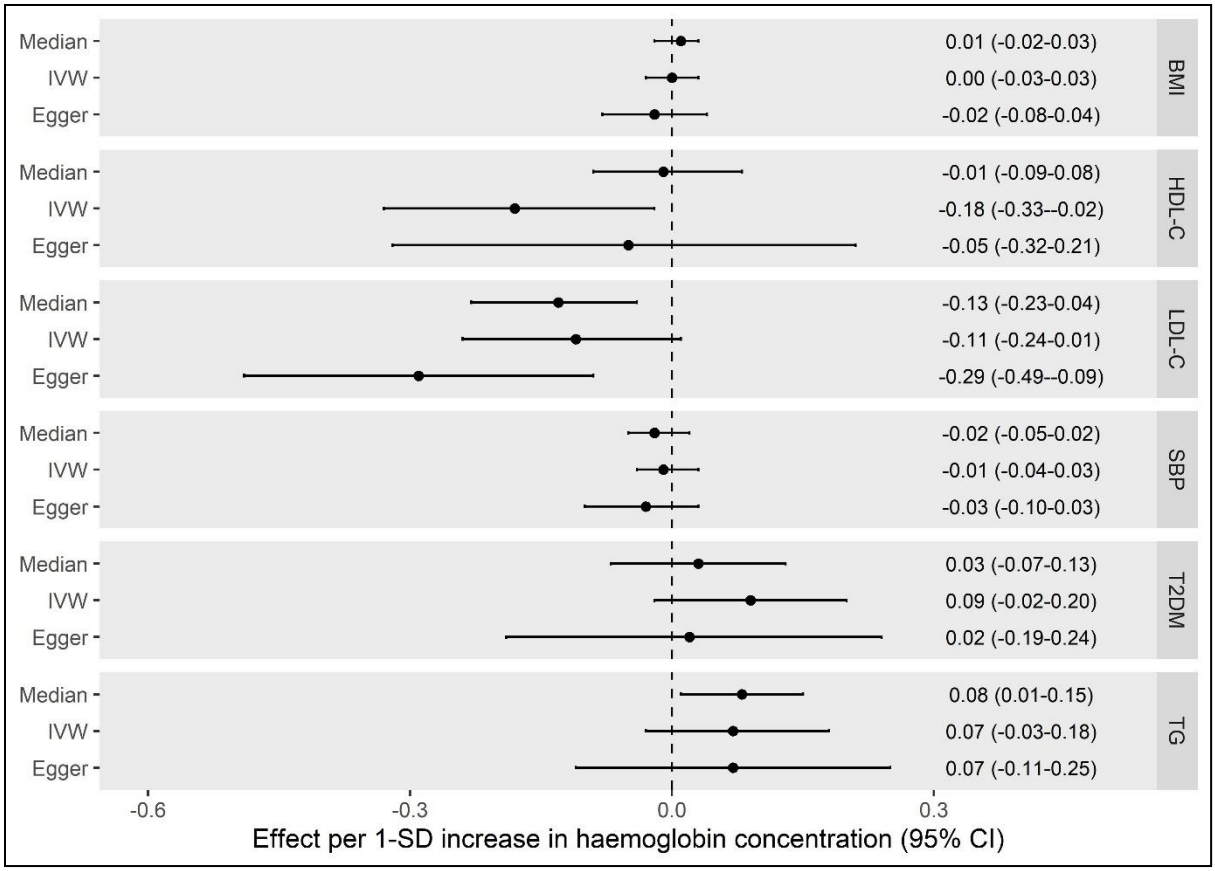

Fig. S8. Mendelian randomization sensitivity analysis results, considering Hb level genetic association estimates that did not adjust for BMI, alcohol consumption or smoking. Effects estimates are given in standard deviation (SD) units for body mass index (BMI), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), systolic blood pressure (SBP) and triglycerides (TG), and in log odds ratio for type 2 diabetes mellitus (T2DM). The Egger intercept *P* values were 0.60 for BMI, 0.26 for HDL-C, 0.03 for LDL-C, 0.37 for SBP, 0.45 for T2DM and 0.97 for TG). IVW: inverse-variance weighted.

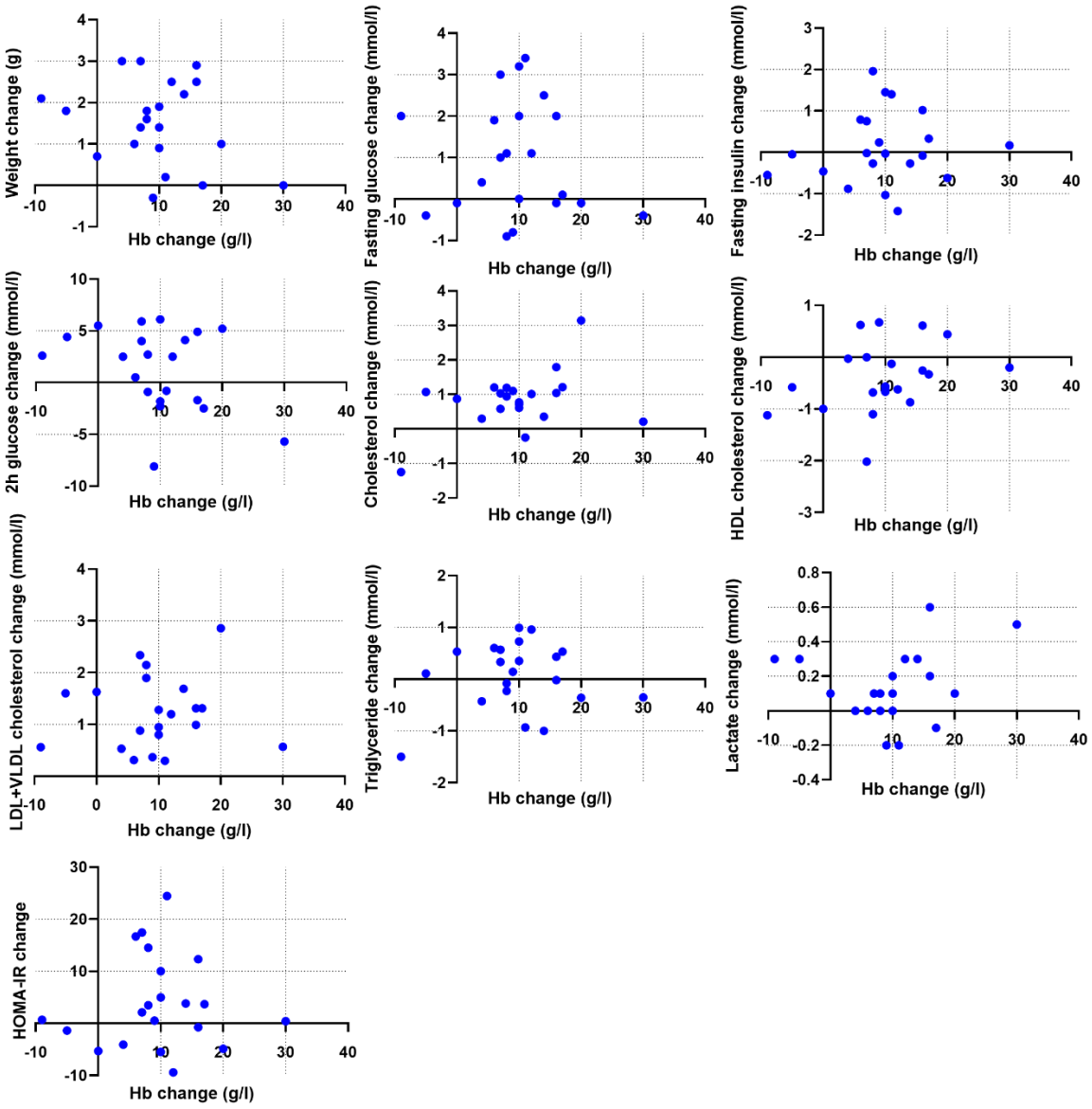

Fig. S9. Correlation of change in Hb levels with changes in body weight, fasting blood glucose, fasting serum insulin, HOMA-IR, 2 h blood glucose levels in GTT, total cholesterol, HDL cholesterol, LDL + VLDL cholesterol, triglycerides and lactate levels, respectively, in individual mice following venesection. The values were determined at baseline and two weeks after venesection. The changes in them in 14 days were correlated. Most data indicate a positive response.

REFERENCES AND NOTES

1. P. van der Harst, W. Zhang, I. Mateo Leach, A. Rendon, N. Verweij, J. Sehmi, D. S. Paul, U. Elling, H. Allayee, X. Li, A. Radhakrishnan, S. T. Tan, K. Voss, C. X. Weichenberger, C. A. Albers, A. Al-Hussani, F. W. Asselbergs, M. Ciullo, F. Danjou, C. Dina, T. Esko, D. M. Evans, L. Franke, M. Gogele, J. Hartiala, M. Hersch, H. Holm, J. J. Hottenga, S. Kanoni, M. E. Kleber, V. Lagou, C. Langenberg, L. M. Lopez, L. P. Lyytikainen, O. Melander, F. Murgia, I. M. Nolte, P. F. O'Reilly, S. Padmanabhan, A. Parsa, N. Pirastu, E. Porcu, L. Portas, I. Prokopenko, J. S. Ried, S. Y. Shin, C. S. Tang, A. Teumer, M. Traglia, S. Ulivi, H. J. Westra, J. Yang, J. H. Zhao, F. Anni, A. Abdellaoui, A. Attwood, B. Balkau, S. Bandinelli, F. Bastardot, B. Benyamin, B. O. Boehm, W. O. Cookson, D. Das, P. I. de Bakker, R. A. de Boer, E. J. de Geus, M. H. de Moor, M. Dimitriou, F. S. Domingues, A. Doring, G. Engstrom, G. I. Eyjolfsson, L. Ferrucci, K. Fischer, R. Galanello, S. F. Garner, B. Genser, Q. D. Gibson, G. Girotto, D. F. Gudbjartsson, S. E. Harris, A. L. Hartikainen, C. E. Hastie, B. Hedblad, T. Illig, J. Jolley, M. Kahonen, I. P. Kema, J. P. Kemp, L. Liang, H. Lloyd-Jones, R. J. Loos, S. Meacham, S. E. Medland, C. Meisinger, Y. Memari, E. Mihailov, K. Miller, M. F. Moffatt, M. Nauck, M. Novatchkova, T. Nutile, I. Olafsson, P. T. Onundarson, D. Parracciani, B. W. Penninx, L. Perseu, A. Piga, G. Pistis, A. Pouta, U. Puc, O. Raitakari, S. M. Ring, A. Robino, D. Ruggiero, A. Ruokonen, A. Saint-Pierre, C. Sala, A. Salumets, J. Sambrook, H. Schepers, C. O. Schmidt, H. H. Sillje, R. Sladek, J. H. Smit, J. M. Starr, J. Stephens, P. Sulem, T. Tanaka, U. Thorsteinsdottir, V. Tragante, W. H. van Gilst, L. J. van Pelt, D. J. van Veldhuisen, U. Volker, J. B. Whitfield, G. Willemsen, B. R. Winkelmann, G. Wirnsberger, A. Algra, F. Cucca, A. P. d'Adamo, J. Danesh, I. J. Deary, A. F. Dominiczak, P. Elliott, P. Fortina, P. Froguel, P. Gasparini, A. Greinacher, S. L. Hazen, M. R. Jarvelin, K. T. Khaw, T. Lehtimaki, W. Maerz, N. G. Martin, A. Metspalu, B. D. Mitchell, G. W. Montgomery, C. Moore, G. Navis, M. Pirastu, P. P. Pramstaller, R. Ramirez-Solis, E. Schadt, J. Scott, A. R. Shuldiner, G. D. Smith, J. G. Smith, H. Snieder, R. Sorice, T. D. Spector, K. Stefansson, M. Stumvoll, W. H. Tang, D. Toniolo, A. Tonjes, P. M. Visscher, P. Vollenweider, N. J. Wareham, B. H. Wolffenbuttel, D. I. Boomsma, J. S. Beckmann, G. V. Dedoussis, P. Deloukas, M. A. Ferreira, S. Sanna, M. Uda, A. A. Hicks, J. M. Penninger, C. Gieger, J. S. Kooner, W. H. Ouwehand, N. Soranzo, J. C. Chambers, Seventy-five genetic loci influencing the human red blood cell. *Nature* **492**, 369–375 (2012).

2. K. V. Patel, Variability and heritability of hemoglobin concentration: An opportunity to improve understanding of anemia in older adults. *Haematologica* **93**, 1281–1283 (2008).

3. L. M. Lobigs, E. J. Knight, Y. O. Schumacher, C. J. Gore, Within-subject haemoglobin variation in elite athletes: A longitudinal investigation of 13 887 haemoglobin concentration readings. *Drug Test. Anal.* **8**, 228–234 (2016).

4. F. F. Jobsis, Noninvasive, infrared monitoring of cerebral and myocardial oxygen sufficiency and circulatory parameters. *Science* **198**, 1264–1267 (1977).

5. E. M. Sevick, B. Chance, J. Leigh, S. Nioka, M. Maris, Quantitation of time- and frequency-resolved optical spectra for the determination of tissue oxygenation. *Anal. Biochem.* **195**, 330–351 (1991).

6. W. G. Kaelin Jr, P. J. Ratcliffe, Oxygen sensing by metazoans: The central role of the HIF hydroxylase pathway. *Mol. Cell* **30**, 393–402 (2008).

7. P. Koivunen, T. Kietzmann, Hypoxia-inducible factor prolyl 4-hydroxylases and metabolism. *Trends Mol. Med.* **24**, 1021–1035 (2018).

8. H. Matsuura, T. Ichiki, E. Inoue, M. Nomura, R. Miyazaki, T. Hashimoto, J. Ikeda, R. Takayanagi, G. H. Fong, K. Sunagawa, Prolyl hydroxylase domain protein 2 plays a critical role in diet-induced obesity and glucose intolerance. *Circulation* **127**, 2078–2087 (2013).

9. L. Rahtu-Korpela, S. Karsikas, S. Hörkkö, R. Blanco Sequeiros, E. Lammentausta, K. A. Mäkelä, K. H. Herzig, G. Walkinshaw, K. I. Kivirikko, J. Myllyharju, R. Serpi, P. Koivunen, HIF prolyl 4-hydroxylase-2 inhibition improves glucose and lipid metabolism and protects against obesity and metabolic dysfunction. *Diabetes* **63**, 3324–3333 (2014).

10. Z. Michailidou, N. M. Morton, J. M. Moreno Navarrete, C. C. West, K. J. Stewart, J. M. Fernandez-Real, C. J. Schofield, J. R. Seckl, P. J. Ratcliffe, Adipocyte pseudohypoxia suppresses lipolysis and facilitates benign adipose tissue expansion. *Diabetes* **64**, 733–745 (2015).

11. P. Rantakallio, Groups at risk in low birth weight infants and perinatal mortality. *Acta Paediatr. Scand.* **193**, Suppl 193:1+ (1969).

12. M. R. Järvelin, U. Sovio, V. King, L. Lauren, B. Xu, M. I. McCarthy, A. L. Hartikainen, J. Laitinen, P. Zitting, P. Rantakallio, P. Elliott, Early life factors and blood pressure at age 31 years in the 1966 northern Finland birth cohort. *Hypertension* **44**, 838–846 (2004).

13. O. T. Raitakari, M. Juonala, T. Rönnemaa, L. Keltikangas-Järvinen, L. Räsänen, M. Pietikäinen, N. Hutri-Kähönen, L. Taittonen, E. Jokinen, J. Marniemi, A. Jula, R. Telama, M. Kähönen, T. Lehtimäki, H. K. Akerblom, J. S. Viikari, Cohort profile: The cardiovascular risk in Young Finns Study. *Int. J. Epidemiol.* **37**, 1220–1226 (2008).

14. P. Wurtz, V. P. Mäkinen, P. Soininen, A. J. Kangas, T. Tukiainen, J. Kettunen, M. J. Savolainen, T. Tammelin, J. S. Viikari, T. Rönnemaa, M. Kähonen, T. Lehtimäki, S. Ripatti, O. T. Raitakari, M. R. Järvelin, M. Ala-Korpela, Metabolic signatures of insulin resistance in 7,098 young adults. *Diabetes* **61**, 1372–1380 (2012).

15. P. Wurtz, Q. Wang, A. J. Kangas, R. C. Richmond, J. Skarp, M. Tiainen, T. Tynkkynen, P. Soininen, A. S. Havulinna, M. Kaakinen, J. S. Viikari, M. J. Savolainen, M. Kähonen, T. Lehtimäki, S. Männistö, S. Blankenberg, T. Zeller, J. Laitinen, A. Pouta, P. Mäntyselkä, M.

Vanhala, P. Elliott, K. H. Pietiläinen, S. Ripatti, V. Salomaa, O. T. Raitakari, M. R. Järvelin, G. D. Smith, M. Ala-Korpela, Metabolic signatures of adiposity in young adults: Mendelian randomization analysis and effects of weight change. *PLOS Med.* **11**, e1001765 (2014).

16. P. Wurtz, A. S. Havulinna, P. Soininen, T. Tynkkynen, D. Prieto-Merino, T. Tillin, A. Ghorbani, A. Artati, Q. Wang, M. Tiainen, A. J. Kangas, J. Kettunen, J. Kaikkonen, V. Mikkilä, A. Jula, M. Kähonen, T. Lehtimäki, D. A. Lawlor, T. R. Gaunt, A. D. Hughes, N. Sattar, T. Illig, J. Adamski, T. J. Wang, M. Perola, S. Ripatti, R. S. Vasan, O. T. Raitakari, R. E. Gerszten, J. P. Casas, N. Chaturvedi, M. Ala-Korpela, V. Salomaa, Metabolite profiling and cardiovascular event risk: A prospective study of 3 population-based cohorts. *Circulation* **131**, 774–785 (2015).

17. B. M. Raabe, J. E. Artwohl, J. E. Purcell, J. Lovaglio, J. D. Fortman, Effects of weekly blood collection in C57BL/6 mice. *J. Am. Assoc. Lab. Anim. Sci.* **50**, 680–685 (2011).

18. A. Laitakari, J. Tapio, K. A. Mäkelä, K. H. Herzig, F. Dengler, H. Gylling, G. Walkinshaw, J. Myllyharju, E. Y. Dimova, R. Serpi, P. Koivunen, HIF-P4H-2 inhibition enhances intestinal fructose metabolism and induces thermogenesis protecting against NAFLD. *J. Mol. Med.* **98**, 719–731 (2020).

19. F. S. Facchini, M. Carantoni, J. Jeppesen, G. M. Reaven, Hematocrit and hemoglobin are independently related to insulin resistance and compensatory hyperinsulinemia in healthy, non-obese men and women. *Metabolism* **47**, 831–835 (1998).

20. M. Cirillo, M. Laurenzi, M. Trevisan, J. Stamler, Hematocrit, blood pressure, and hypertension. The Gubbio Population Study. *Hypertension* **20**, 319–326 (1992).

21. L. E. Böttiger, L. A. Carlson, Relation between serum cholesterol and triglyceride concentration and haemoglobin values in non-anaemic healthy persons. *Br. Med. J.* **3**, 731–733 (1972).

22. P. Hämäläinen, J. Saltevo, H. Kautiainen, P. Mäntyselkä, M. Vanhala, Erythropoietin, ferritin, haptoglobin, hemoglobin and transferrin receptor in metabolic syndrome: A case control study. *Cardiovasc. Diabetol.* **11**, 116 (2012).

23. P. Hämäläinen, J. Saltevo, H. Kautiainen, P. Mäntyselkä, M. Vanhala, Hemoglobin level and lipoprotein particle size. *Lipids Health Dis.* **17**, 10 (2018).

24. Y. Hashimoto, M. Tanaka, T. Kimura, N. Kitagawa, M. Hamaguchi, M. Asano, M. Yamazaki, Y. Oda, H. Toda, N. Nakamura, M. Fukui, Hemoglobin concentration and incident metabolic syndrome: A population-based large-scale cohort study. *Endocrine* **50**, 390–396 (2015).

25. S. G. Wannamethee, I. J. Perry, A. G. Shaper, Hematocrit and risk of NIDDM. *Diabetes* **45**, 576–579 (1996).

26. J. D. Voss, P. Masuoka, B. J. Webber, A. I. Scher, R. L. Atkinson, Association of elevation, urbanization and ambient temperature with obesity prevalence in the United States. *Int. J. Obes.* **37**, 1407–1412 (2013).

27. O. O. Woolcott, M. Ader, R. N. Bergman, Glucose homeostasis during short-term and prolonged exposure to high altitudes. *Endocr. Rev.* **36**, 149–173 (2015).

28. K. L. Marlatt, F. L. Greenway, J. Kyle Schwab, E. Ravussin, Two weeks of moderate hypoxia improves glucose tolerance in individuals with type 2 diabetes. *Int. J. Obes.* **44**, 744–747 (2020).

29. N. Chen, C. Hao, X. Peng, H. Lin, A. Yin, L. Hao, Y. Tao, X. Liang, Z. Liu, C. Xing, J. Chen, L. Luo, L. Zuo, Y. Liao, B. C. Liu, R. Leong, C. Wang, C. Liu, T. Neff, L. Szczech, K. P. Yu, Roxadustat for anemia in patients with kidney disease not receiving dialysis. *N. Engl. J. Med.* **381**, 1001–1010 (2019).

30. C. V. Dang, The interplay between MYC and HIF in the Warburg effect. *Ernst Schering Found. Symp. Proc.* **4**, 35–53 (2007).

31. P. Soininen, A. J. Kangas, P. Wurtz, T. Tukiainen, T. Tynkkynen, R. Laatikainen, M. R. Järvelin, M. Kähönen, T. Lehtimäki, J. Viikari, O. T. Raitakari, M. J. Savolainen, M. Ala-Korpela, High-throughput serum NMR metabonomics for cost-effective holistic studies on systemic metabolism. *Analyst* **134**, 1781–1785 (2009).

32. M. Matsuda, Measuring and estimating insulin resistance in clinical and research settings. *Nutr. Metab. Cardiovasc. Dis.* **20**, 79–86 (2010).

33. A. Subramanian, P. Tamayo, V. K. Mootha, S. Mukherjee, B. L. Ebert, M. A. Gillette, A. Paulovich, S. L. Pomeroy, T. R. Golub, E. S. Lander, J. P. Mesirov, Gene set enrichment analysis: A knowledge-based approach for interpreting genome-wide expression profiles. *Proc. Natl. Acad. Sci. U.S.A.* **102**, 15545–15550 (2005).

34. E. Raitoharju, I. Seppälä, N. Oksala, L. P. Lyytikäinen, O. Raitakari, J. Viikari, M. Ala-Korpela, P. Soininen, A. J. Kangas, M. Waldenberger, N. Klopp, T. Illig, J. Leiviskä, B. M. Loo, N. Hutri-Kähönen, M. Kähönen, R. Laaksonen, T. Lehtimäki, Blood microRNA profile associates with the levels of serum lipids and metabolites associated with glucose metabolism and insulin resistance and pinpoints pathways underlying metabolic syndrome: The cardiovascular risk in Young Finns Study. *Mol. Cell. Endocrinol.* **391**, 41–49 (2014).

35. W. J. Astle, H. Elding, T. Jiang, D. Allen, D. Ruklisa, A. L. Mann, D. Mead, H. Bouman, F. Riveros-Mckay, M. A. Kostadima, J. J. Lambourne, S. Sivapalaratnam, K. Downes, K.

Kundu, L. Bomba, K. Berentsen, J. R. Bradley, L. C. Daugherty, O. Delaneau, K. Freson, S. F. Garner, L. Grassi, J. Guerrero, M. Haimel, E. M. Janssen-Megens, A. Kaan, M. Kamat, B. Kim, A. Mandoli, J. Marchini, J. H. A. Martens, S. Meacham, K. Megy, J. O'Connell, R. Petersen, N. Sharifi, S. M. Sheard, J. R. Staley, S. Tuna, M. van der Ent, K. Walter, S. Y. Wang, E. Wheeler, S. P. Wilder, V. Iotchkova, C. Moore, J. Sambrook, H. G. Stunnenberg, E. Di Angelantonio, S. Kaptoge, T. W. Kuijpers, E. Carrillo-de-Santa-Pau, D. Juan, D. Rico, A. Valencia, L. Chen, B. Ge, L. Vasquez, T. Kwan, D. Garrido-Martin, S. Watt, Y. Yang, R. Guigo, S. Beck, D. S. Paul, T. Pastinen, D. Bujold, G. Bourque, M. Frontini, J. Danesh, D. J. Roberts, W. H. Ouwehand, A. S. Butterworth, N. Soranzo, The allelic landscape of human blood cell trait variation and links to common complex disease. *Cell* **167**, 1415–1429.e19 (2016).

36. G. Hemani, J. Zheng, B. Elsworth, K. H. Wade, V. Haberland, D. Baird, C. Laurin, S. Burgess, J. Bowden, R. Langdon, V. Y. Tan, J. Yarmolinsky, H. A. Shihab, N. J. Timpson, D. M. Evans, C. Relton, R. M. Martin, G. Davey Smith, T. R. Gaunt, P. C. Haycock, The MR-Base platform supports systematic causal inference across the human phenome. *eLife* **7**, e34408 (2018).

37. S. L. Pulit, C. Stoneman, A. P. Morris, A. R. Wood, C. A. Glastonbury, J. Tyrrell, L. Yengo, T. Ferreira, E. Marouli, Y. Ji, J. Yang, S. Jones, R. Beaumont, D. C. Croteau-Chonka, T. W. Winkler; GIANT Consortium, A. T. Hattersley, R. J. F. Loos, J. N. Hirschhorn, P. M. Visscher, T. M. Frayling, H. Yaghootkar, C. M. Lindgren, Meta-analysis of genome-wide association studies for body fat distribution in 694 649 individuals of European ancestry. *Hum. Mol. Genet.* **28**, 166–174 (2019).

38. Global Lipids Genetics Consortium, Discovery and refinement of loci associated with lipid levels. *Nat. Genet.* **45**, 1274–1283 (2013).

39. A. R. Carter, D. Gill, N. M. Davies, A. E. Taylor, T. Tillmann, J. Vaucher, R. E. Wootton, M. R. Munafo, G. Hemani, R. Malik, S. Seshadri, D. Woo, S. Burgess, G. Davey Smith, M. V. Holmes, I. Tzoulaki, L. D. Howe, A. Dehghan, Understanding the consequences of education inequality on cardiovascular disease: Mendelian randomisation study. *BMJ* **365**, l1855 (2019).

40. A. Mahajan, D. Taliun, M. Thurner, N. R. Robertson, J. M. Torres, N. W. Rayner, A. J. Payne, V. Steinthorsdottir, R. A. Scott, N. Grarup, J. P. Cook, E. M. Schmidt, M. Wuttke, C. Sarnowski, R. Magi, J. Nano, C. Gieger, S. Trompet, C. Lecoeur, M. H. Preuss, B. P. Prins, X. Guo, L. F. Bielak, J. E. Below, D. W. Bowden, J. C. Chambers, Y. J. Kim, M. C. Y. Ng, L. E. Petty, X. Sim, W. Zhang, A. J. Bennett, J. Bork-Jensen, C. M. Brummett, M. Canouil, K. U. Ec Kardt, K. Fischer, S. L. R. Kardia, F. Kronenberg, K. Lall, C. T. Liu, A. E. Locke, J. Luan, I. Ntalla, V. Nylander, S. Schonherr, C. Schurmann, L. Yengo, E. P. Bottinger, I. Brandslund, C. Christensen, G. Dedoussis, J. C. Florez, I. Ford, O. H. Franco, T. M.

Frayling, V. Giedraitis, S. Hackinger, A. T. Hattersley, C. Herder, M. A. Ikram, M. Ingelsson, M. E. Jorgensen, T. Jorgensen, J. Kriebel, J. Kuusisto, S. Ligthart, C. M. Lindgren, A. Linneberg, V. Lyssenko, V. Mamakou, T. Meitinger, K. L. Mohlke, A. D. Morris, G. Nadkarni, J. S. Pankow, A. Peters, N. Sattar, A. Stancakova, K. Strauch, K. D. Taylor, B. Thorand, G. Thorleifsson, U. Thorsteinsdottir, J. Tuomilehto, D. R. Witte, J. Dupuis, P. A. Peyser, E. Zeggini, R. J. F. Loos, P. Froguel, E. Ingelsson, L. Lind, L. Groop, M. Laakso, F. S. Collins, J. W. Jukema, C. N. A. Palmer, H. Grallert, A. Metspalu, A. Dehghan, A. Kottgen, G. R. Abecasis, J. B. Meigs, J. I. Rotter, J. Marchini, O. Pedersen, T. Hansen, C. Langenberg, N. J. Wareham, K. Stefansson, A. L. Gloyn, A. P. Morris, M. Boehnke, M. I. McCarthy, Fine-mapping type 2 diabetes loci to single-variant resolution using high-density imputation and islet-specific epigenome maps. *Nat. Genet.* **50**, 1505–1513 (2018).

41. S. Burgess, A. Butterworth, S. G. Thompson, Mendelian randomization analysis with multiple genetic variants using summarized data. *Genet. Epidemiol.* **37**, 658–665 (2013).

42. S. Burgess, J. Bowden, T. Fall, E. Ingelsson, S. G. Thompson, Sensitivity analyses for robust causal inference from Mendelian randomization analyses with multiple genetic variants. *Epidemiology* **28**, 30–42 (2017).

43. J. Bowden, G. Davey Smith, P. C. Haycock, S. Burgess, Consistent estimation in Mendelian randomization with some invalid instruments using a weighted median estimator. *Genet. Epidemiol.* **40**, 304–314 (2016).

44. J. Bowden, G. Davey Smith, S. Burgess, Mendelian randomization with invalid instruments: Effect estimation and bias detection through Egger regression. *Int. J. Epidemiol.* **44**, 512–525 (2015).

45. GTEx Consortium; Laboratory, Data Analysis &Coordinating Center (LDACC)—Analysis Working Group; Statistical Methods groups—Analysis Working Group; Enhancing GTEx (eGTEx) groups; NIH Common Fund; NIH/NCI; NIH/NHGRI; NIH/NIMH; NIH/NIDA; Biospecimen Collection Source Site—NDRI; Biospecimen Collection Source Site—RPCI; Biospecimen Core Resource—VARI; Brain Bank Repository—University of Miami Brain Endowment Bank; Leidos Biomedical—Project Management; ELSI Study; Genome Browser Data Integration &Visualization—EBI; Genome Browser Data Integration &Visualization—UCSC Genomics Institute, University of California Santa Cruz; Lead analysts; Laboratory, Data Analysis &Coordinating Center (LDACC); NIH program management; Biospecimen collection; Pathology; eQTL manuscript working group; A. Battle, C. D. Brown, B. E. Engelhardt, S. B. Montgomery, Genetic effects on gene expression across human tissues. *Nature* **550**, 204–213 (2017).

46. O. O. Yavorska, S. Burgess, MendelianRandomization: An R package for performing Mendelian randomization analyses using summarized data. *Int. J. Epidemiol.* **46**, 1734–1739 (2017).

47. H. Wieland, D. Seidel, A simple specific method for precipitation of low density lipoproteins. *J. Lipid Res.* **24**, 904–909 (1983).

48. H. Sugiuchi, Y. Uji, H. Okabe, T. Irie, K. Uekama, N. Kayahara, K. Miyauchi, Direct measurement of high-density lipoprotein cholesterol in serum with polyethylene glycol-modified enzymes and sulfated alpha-cyclodextrin. *Clin. Chem.* **41**, 717–723 (1995).

49. M. Matsuda, R. A. DeFronzo, Insulin sensitivity indices obtained from oral glucose tolerance testing: Comparison with the euglycemic insulin clamp. *Diabetes Care* **22**, 1462–1470 (1999).

50. P. Wurtz, A. J. Kangas, P. Soininen, D. A. Lawlor, G. Davey Smith, M. Ala-Korpela, Quantitative serum nuclear magnetic resonance metabolomics in large-scale epidemiology: A primer on -omic technologies. *Am. J. Epidemiol.* **186**, 1084–1096 (2017).

51. T. Tammelin, S. Näyhä, H. Rintamäki, Cardiorespiratory fitness of males and females of northern Finland birth cohort of 1966 at age 31. *Int. J. Sports Med.* **25**, 547–552 (2004).

52. M. C. Bosco, M. Puppo, C. Santangelo, L. Anfosso, U. Pfeffer, P. Fardin, F. Battaglia, L. Varesio, Hypoxia modifies the transcriptome of primary human monocytes: Modulation of novel immune-related genes and identification of CC-chemokine ligand 20 as a new hypoxia-inducible gene. *J. Immunol.* **177**, 1941–1955 (2006).

53. N. J. Cox, Speaking Stata: Graphing model diagnostics. *Stata J.* **4**, 449–475 (2004).

54. Q. Wang, P. Wurtz, K. Auro, L. Morin-Papunen, A. J. Kangas, P. Soininen, M. Tiainen, T. Tynkkynen, A. Joensuu, A. S. Havulinna, K. Aalto, M. Salmi, S. Blankenberg, T. Zeller, J. Viikari, M. Kähönen, T. Lehtimäki, V. Salomaa, S. Jalkanen, M. R. Järvelin, M. Perola, O. T. Raitakari, D. A. Lawlor, J. Kettunen, M. Ala-Korpela, Effects of hormonal contraception on systemic metabolism: Cross-sectional and longitudinal evidence. *Int. J. Epidemiol.* **45**, 1445–1457 (2016).

55. P. Wurtz, Q. Wang, P. Soininen, A. J. Kangas, G. Fatemifar, T. Tynkkynen, M. Tiainen, M. Perola, T. Tillin, A. D. Hughes, P. Mäntyselkä, M. Kähönen, T. Lehtimäki, N. Sattar, A. D. Hingorani, J. P. Casas, V. Salomaa, M. Kivimäki, M. R. Järvelin, G. Davey Smith, M. Vanhala, D. A. Lawlor, O. T. Raitakari, N. Chaturvedi, J. Kettunen, M. Ala-Korpela, Metabolomic profiling of statin use and genetic inhibition of HMG-CoA reductase. *J. Am. Coll. Cardiol.* **67**, 1200–1210 (2016).

56. J. Pinheiro, D. Bates, S. DebRoy, D. Sarkar, R Core Team, *nlme: Linear and Nonlinear Mixed Effects Models*. R package version 3.1–128 (2016); http://CRAN.R-project.org/package=nlme.

57. H. C. Holtkamp, N. J. Verhoef, B. Leijnse, The difference between the glucose concentrations in plasma and whole blood. *Clin. Chim. Acta* **59**, 41–49 (1975).

58. G. M. Fitzmaurice, N. M. Laird, J. H. Ware, *Applied Longitudinal Analysis* (John Wiley and Sons, ed. 2, 2011).