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Age- and Sex-Specific Causal Effects of Adiposity on Cardiovascular Risk Factors

Observational studies have reported different effects of adiposity on cardiovascular risk factors across age and sex. Since cardiovascular risk factors are enriched in obese individuals, it has not been easy to dissect the effects of adiposity from those of other risk factors. We used a Mendelian randomization approach, applying a set of 32 genetic markers to estimate the causal effect of adiposity on blood pressure, glycemic indices, circulating lipid levels, and markers of inflammation and liver disease in up to 67,553 individuals. All analyses were stratified by age (cutoff 55 years of age) and sex. The genetic score was associated with BMI in both nonstratified analysis ($P = 2.8 \times 10^{-107}$) and stratified analyses (all $P < 3.3 \times 10^{-30}$). We found evidence of a causal effect of adiposity on blood pressure, fasting levels of insulin, C-reactive protein, interleukin-6, HDL cholesterol, and triglycerides in a nonstratified analysis and in the <55-year stratum. Further, we found evidence of a smaller causal effect on total cholesterol (P for difference = 0.015) in the ≥55-year stratum than in the <55-year stratum, a finding that could be explained by biology, survival bias, or differential medication. In conclusion, this study extends previous knowledge of the effects of adiposity by providing sex- and age-specific causal estimates on cardiovascular risk factors.

The incidence of overweight and obesity is increasing rapidly on a global level. Adiposity constitutes an important risk factor for cardiovascular disease (CVD), which is the major cause of morbidity and mortality for both men and women (1), although CVD generally occurs later in

women than in men (2,3). There is strong evidence from randomized clinical trials (4,5) that weight loss induced by dietary change reduces the levels of cardiovascular risk factors, such as blood pressure and circulating lipid levels. However, intervention studies of weight loss are often difficult to interpret because the intervention may affect several separate pathways. For example, bariatric surgery can affect glucose metabolism through pathways other than weight loss (6). We and others (7,8) have previously applied Mendelian randomization methods to assess and confirm the causal role of adiposity in cardiometabolic disease. In Mendelian randomization study designs, one

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or several genetic variants, usually single nucleotide polymorphisms (SNPs), associated with a modifiable risk factor are used as instrumental variables (IVs). The IVs provide unbiased estimates of the causal relation of the exposure to the risk factor (here, adiposity) with an outcome of interest (here, other cardiovascular risk factors) (9).

Age and sex differences in adiposity and body fat distribution, as well as physiology of the heart and blood vessels, are evident even before overt CVD becomes evident (10,11). As an example, men are usually less sensitive to insulin than women, given a certain BMI, which may partly be explained by differences in fat distribution (12). Moreover, the strength of association between obesity and a number of cardiovascular risk factors, such as blood pressure (13–15) and total serum cholesterol (16) and fasting glucose (17) levels, has been reported to decline with age. The role of weight loss as a health-promoting intervention in older age groups has been debated lately as large studies (18) have shown that the excess mortality from obesity markedly declines with age and that the risk of sarcopenia and osteoporosis increases with weight loss. Improved understanding of the effect of adiposity in different age and sex groups may advance prevention of CVD through the possibility of efforts directed toward groups with the largest causal effect. However, stratified observational studies suffer from the risk of confounding and reverse causation, problems that can be avoided using a Mendelian randomization design.

The objective of the current study was to use a Mendelian randomization design to assess whether adiposity causally affects known cardiovascular risk factors at a similar magnitude in men and women and before and after 55 years of age.

RESEARCH DESIGN AND METHODS

Study Population
Population-based studies of European ancestry enrolled in the European Network for Genetic and Genomic Epidemiology (ENGAGE) Consortium were invited to participate in the study. In total, 30 studies contributed to the primary and/or secondary analysis in the current study (Supplementary Table 1). A standardized analysis plan was provided to all partners, and individual-level analyses were performed at each center separately. The distributions of age, BMI, and sex in the participating cohorts are shown in Supplementary Table 2.

Genetic Instruments
An individual nonweighted genetic risk score using the 32 SNPs ("lead SNPs") reported by Speliotes et al. (19) was used as the IV in 25 studies for the main analyses (listed in Supplementary Table 3). The score was created by summing the number of BMI-increasing alleles (0, 1, 2) for each of the 32 SNPs. When direct genotype data were not available for an SNP in a particular study, we prioritized as follows: 1) imputed allele dosage data with high imputation quality (IMPUTE proper_info$\geq$0.4 or MACH r^2hat$\geq$0.3) (20); 2)

information from directly genotyped proxy SNPs from a predefined list of variants that are in high linkage disequilibrium (LD) with lead SNPs; or 3) imputation to a prespecified allele dosage (2*reported effect allele frequency from the study by Speliotes et al. [19]). Eighteen of the studies included in the genetic score analyses had directly genotyped or high-quality imputed genotype information on all SNPs, while seven studies lacked information on three SNPs each and used the prespecified allele dosage for those three SNPs.

In secondary analyses, we used one SNP as the IV to maximize the study sample (five additional ENGAGE studies had information on this SNP only, for a total of 30 studies). For this instrument, we used direct genotype information for the *FTO* variant rs9939609 from participating studies when possible (16 studies). If rs9939609 was not genotyped directly, we prioritized as follows: 1) the HapMap II CEU (European) reference panel imputed genetic information (hapmap.ncbi.nlm.nih.gov) for rs9939609 (six studies) or 2) genotype information from a predefined list of proxies that are in high LD with rs9939609. We used the directly genotyped proxies rs11075989 (four studies, r^2 with rs9939609 = 1.0 in HapMap II), rs3751812 (one study, r^2 = 1.0), rs1558902 (one study, r^2 = 0.93), and rs1421085 (two studies, r^2 = 0.93). We estimated the effects of the BMI-increasing A allele of rs9939609 or for the corresponding alleles from proxies (using HapMap II CEU LD data) on BMI and cardiovascular risk factors.

Individuals were excluded from analysis when the overall genotyping array sample call rate was <95%. All studies reported SNPs with Hardy-Weinberg equilibrium exact P values >0.0001 and a call rate >0.95 for genotyped SNPs.

Outcomes
We studied the following quantitative outcomes: 1) diastolic and systolic blood pressure where observed values were increased with 15 mmHg for systolic blood pressure and 10 mmHg for diastolic blood pressure in case of reported hypertension medication; 2) circulating lipid fraction (only in individuals not receiving lipid-lowering medication) (concentrations of HDL cholesterol [HDL-C], LDL cholesterol [LDL-C], total cholesterol, and triglycerides); 3) measurements of glucose homeostasis in nondiabetic subjects using the following glycemic traits: concentrations of fasting glucose, 2-h glucose from the oral glucose tolerance test (OGTT), hemoglobin A_{1c} (HbA$_{1c}$), and fasting insulin; 4) liver enzyme activity (alanine aminotransferase [ALT], γ-glutamyl transferase [GGT]); and 5) inflammation markers (concentrations of C-reactive protein [CRP] and interleukin-6 [IL-6]). The measurement methods used in each study and the respective matrix are reported in Supplementary Tables 4, 5, and 6. The following variables were transformed to the natural logarithmic scale prior to further analysis due to their skewed distribution when examining normal probability plots in preliminary analysis (levels of fasting insulin, ALT, GGT,

CRP, IL-6, and triglycerides). All quantitative phenotypes including BMI were z score (SD) standardized prior to further analysis to reduce heterogeneity across studies and to make it possible to compare the adiposity effect between outcomes.

Statistical Analysis

Association Analyses
We evaluated associations of BMI and IVs with each outcome separately, as well as associations between IVs and BMI. For all analyses, we used linear regressions in each study separately, assuming an additive effect of the number of BMI-increasing alleles. The associations between BMI and outcomes are hereafter referred to as "observational." All analyses were performed in the full study sample, as well as stratified by age at measurement (cutoff 55 years of age), and in men and women separately. If measurements were available both before and after age 55 years for the same individuals, these individuals were included in both age strata (using the respective measurement). Regression analyses were adjusted for age and sex (only in the sex-pooled analysis) by including age at measurement and sex as covariates. Individual studies were adjusted for additional study-specific covariates such as the study center when relevant. Random-effects meta-analysis (21,22) of the study-specific results was performed using the *rmeta* package, in R version 3.0.0.

IV Analyses
We used IV estimators to quantify the strength of the causal associations between adiposity and other cardiovascular risk factors. The estimator (β_{IV}) was calculated as the ratio between the two regression coefficients determined from association meta-analyses in each stratum (Eq. 1): estimated genetic score or *FTO* effect on the given outcome ($\beta_{\text{genetic instrument\_outcome}}$), and estimated genetic score or *FTO* effect on BMI ($\beta_{\text{genetic instrument\_BMI}}$).

$$\beta_{IV} = \frac{\beta_{\text{genetic instrument\_outcome}}}{\beta_{\text{genetic instrument\_BMI}}} \qquad (1)$$

For quantitative outcomes with a single instrument, the IV estimator derived by Equation 1 is identical to that derived by the widely used two-stage least squares method (23). The SEs for the IV estimators were calculated using the delta method (Eq. 2), which we have previously evaluated for this purpose (7):

$$SE_{IV} =$$
$$abs(\beta_{IV}) \sqrt{\left(\frac{SE_{\text{genetic instrument\_BMI}}}{\beta_{\text{genetic instrument\_BMI}}}\right)^2 + \left(\frac{SE_{\text{genetic instrument\_outcome}}}{\beta_{\text{genetic instrument\_outcome}}}\right)^2}$$
$$(2)$$

For each trait, we tested the null hypothesis of no difference between the IV estimator and the observational regression-based estimator via the z test. We also tested differences between estimates for men and women and differences between the two age strata using the same approach.

Multiple Testing Correction
We used the Benjamini and Hochberg (24) procedure implemented in the STATA *multproc* procedure to correct the level of significance to a 5% false discovery rate (FDR). The procedure was applied to all 266 P values in the main analysis together.

RESULTS

Association of the Genetic Score With BMI
Random-effects meta-analysis of the association between the genetic score and BMI in 25 studies ($n = 81,764$) showed a positive effect (0.030 SD units of BMI per additional allele [95% CI 0.028–0.033], $P = 2.8 \times 10^{-107}$). Stratum-specific point estimates were similar to those of the pooled data, and no differences between strata were found using the z test (Table 1).

Associations of BMI With Cardiovascular Risk Factors
We applied the FDR procedure to correct for all 266 tests reported in Tables 2, 3, and 4. At a 5% FDR level, the corrected critical P value was estimated to 0.022, which should be considered as the P value threshold to denote statistical significance in this report.

The random-effects meta-analysis showed that BMI was associated with all quantitative phenotypes in all strata (Tables 2, 3, and 4, observational analysis) with the exception of the ≥55-year stratum, where no association was found with LDL-C and total cholesterol levels (Table 3).

Instrumental Variable Analysis Using the Genetic Score
Nonstratified IV analysis using the genetic score showed evidence for causal effects of adiposity on the following: *1*) diastolic and systolic blood pressure (after adjustment for blood pressure medication); *2*) levels of HDL-C and triglycerides (in individuals not receiving lipid-lowering medication); *3*) fasting levels of insulin; and *4*) levels of CRP and IL-6 (Table 2).

Stratified IV analysis showed consistent evidence for causal effects of adiposity in all age and sex strata for HDL-C. For the other outcomes, we found evidence of causality in up to three of the strata (Tables 3 and 4).

Differences Between Age Strata for the Observational and IV Analyses of the Association of Adiposity With Outcomes
We observed significantly larger regression coefficients in the observational analysis for BMI in the <55-year stratum than in the ≥55-year stratum for 6 of the 14 outcomes, as follows: diastolic blood pressure and levels of LDL-C, total cholesterol, triglycerides, GGT, and CRP. We found a smaller regression coefficient for the association of BMI with fasting glucose levels in the <55-year stratum (Table 3).

In line with the observational analysis, for these six outcomes all IV point estimates were higher in the <55-year stratum than in the ≥55-year stratum. However, these differences were nonsignificant, except for the total cholesterol analysis ($P = 0.015$).

Differences Between Sex Strata for the Observational and IV Analyses of the Association of Adiposity With Outcomes

In observational analyses, we observed larger regression coefficients in men than in women for associations of BMI with systolic and diastolic blood pressure and levels of total cholesterol, triglycerides, ALT, GGT, and fasting insulin. In line with the observational analysis for these seven outcomes, all IV point estimates were consistently higher (but not significantly different) in men than in women, with the exception of triglycerides and ALT. There were larger effects of adiposity on CRP levels in women than in men in the observational analysis, and, in line with this, the IV point estimate was higher for women, but again was not significantly different (Table 4).

Differences Between Observational Analysis and IV Analysis

We found the IV estimates for diastolic blood pressure and fasting glucose level to be significantly reduced or abolished compared with the observational estimates in the ≥55-year stratum (Table 3). This finding implies the presence of confounding of the observational results for these outcomes in the old stratum, which was not as prominent in the young stratum. We also found that the IV was significantly smaller than the observational estimate for fasting insulin levels, both in nonstratified analysis and for <55-year stratum. Further, the IV estimates in women were found to be significantly smaller than the observational estimates for diastolic blood pressure.

Instrumental Variable Analysis Using *FTO*

In secondary analyses, we performed random-effects meta-analysis of the association between *FTO* variant and BMI in the 30 studies ($n = 141,800$) that showed a positive effect of the A-allele of rs9939609 on BMI (0.081 SD units per additional A-allele [95% CI 0.070–0.091], $P = 1.4 \times 10^{-50}$) (Table 1). The results using *FTO* as an IV are presented in Supplementary Tables 7, 8, and 9. Overall, the results were similar in directions and effect sizes to those using the genetic score. Because the sample size was much larger for the *FTO* instrument for some outcomes, the *FTO* IV estimates were in some instances more precise (smaller SEs) with respect to the estimates using the genetic score. In addition to the findings presented above, the *FTO* analysis provided evidence of a causal effect of adiposity on ALT activity. Further, the IV estimate using *FTO* was significantly higher for men than for women for fasting insulin level ($P = 0.01$), a result that is consistent with the observational estimate (Supplementary Table 9).

Table 1—Association of genetic score and *FTO* variant with BMI

	Genetic score						*FTO*					
	n_{stud}	n	β (95% CI)	P	F	P_{diff}	n_{stud}	n	β (95% CI)	P	F	P_{diff}
All	25	81,764	0.030 (0.028–0.033)	2.80×10^{-107}	484	NA	30	141,800	0.081 (0.070–0.091)	1.40×10^{-50}	224	NA
Men	24	38,704	0.029 (0.025–0.032)	3.63×10^{-56}	249	0.19	29	68,971	0.075 (0.062–0.087)	7.60×10^{-31}	133	0.28
Women	24	43,060	0.032 (0.029–0.035)	1.06×10^{-97}	440		29	72,829	0.084 (0.072–0.096)	2.00×10^{-44}	195	
≥55-years-old group	21	37,105	0.027 (0.023–0.032)	3.28×10^{-30}	130	0.14	25	52,057	0.076 (0.061–0.091)	1.90×10^{-23}	100	0.39
<55-years-old group	23	45,775	0.032 (0.029–0.034)	1.60×10^{-119}	540		28	90,855	0.084 (0.071–0.097)	9.70×10^{-38}	165	

β (95% CI), per-allele effect on BMI on SD scale; n, total number of individuals in the analysis; n_{stud}, number of studies in analysis; P_{diff}, P value for test of differences between strata.

Table 2—Nonstratified observational and IV analyses based on a genetic score of the association of adiposity with cardiovascular risk factors

	Observational analyses			IV analyses			
	n	β (95% CI)	P^*	n	β (95% CI)	P^*	$P_{IV\text{-}obs}^*$
Diastolic blood pressure	117,230	0.25 (0.23–0.27)	2.5×10^{-136}	66,997	0.15 (0.03–0.26)	0.01	0.08
Systolic blood pressure	117,781	0.22 (0.20–0.24)	2.3×10^{-101}	67,553	0.16 (0.04–0.28)	0.01	0.31
Fasting glucose	69,110	0.19 (0.15–0.22)	2.9×10^{-26}	37,181	0.09 (−0.01 to 0.19)	0.07	0.06
2-h post-OGTT glucose	22,204	0.19 (0.11–0.27)	5.9×10^{-6}	4,596	0.11 (−0.19 to 0.40)	0.48	0.61
HbA$_{1c}$	29,451	0.16 (0.11–0.21)	5.1×10^{-10}	26,901	0.19 (0.01–0.36)	0.03	0.79
ln fasting insulin	40,165	0.48 (0.44–0.52)	1.2×10^{-127}	24,614	0.31 (0.17–0.45)	9.6×10^{-6}	0.02
ln CRP	71,960	0.33 (0.30–0.36)	2.7×10^{-102}	47,781	0.30 (0.20–0.41)	8.8×10^{-9}	0.56
ln IL-6	11,878	0.13 (0.06–0.19)	2.0×10^{-4}	10,285	0.26 (0.08–0.45)	0.01	0.18
HDL-C	93,015	−0.28 (−0.29 to −0.26)	5.8×10^{-211}	58,387	−0.33 (−0.42 to −0.24)	1.1×10^{-12}	0.29
LDL-C	86,793	0.11 (0.08–0.14)	5.7×10^{-17}	52,858	0.08 (−0.02 to 0.19)	0.11	0.64
Total cholesterol	107,741	0.08 (0.06–0.10)	9.7×10^{-15}	59,329	0.00 (−0.11 to 0.11)	0.97	0.17
ln triglycerides	101,709	0.31 (0.30–0.32)	$<1 \times 10^{-300}$	54,417	0.23 (0.12–0.34)	2.7×10^{-5}	0.18
ln ALT	45,473	0.23 (0.19–0.26)	6.5×10^{-31}	23,927	0.09 (−0.09 to 0.26)	0.32	0.13
ln GGT	71,859	0.22 (0.20–0.24)	4.3×10^{-87}	26,831	0.15 (0.00–0.29)	0.04	0.35

All models were adjusted for sex and age. β (95% CI), effect per SD change of BMI on trait (SD scale); $P_{IV\text{-}obs}$, P value from test of difference between observational and IV analyses. *A nominal P value of <0.022 is considered significant at a 5% FDR level.

DISCUSSION

In this Mendelian randomization study, we extended previous efforts to assess causal effects of adiposity on cardiovascular risk factors by examining this relation across age and sex groups. We based our estimations on associations of genetic instruments with 14 cardiovascular risk factors in up to 67,553 individuals in primary analyses and 116,443 individuals in secondary analyses. We provide evidence for causal effects of adiposity on blood pressure (systolic and diastolic), circulating levels of lipids (HDL-C, triglycerides), glucose homeostasis (fasting insulin levels), and markers of inflammation (CRP, IL-6). Similar to what was seen in the observational analysis, causal estimates for the effect of adiposity on markers of inflammation, liver damage, lipid levels, and blood pressure were consistently larger in the <55-year stratum than in the ≥55-year stratum, although most of these differences were not statistically significant in the IV analyses. Further, in IV analyses, the point estimates of the effect of adiposity on systolic and diastolic blood pressure were larger in men than in women. For markers of inflammation, we observed the reverse (i.e., larger causal estimates in women than in men). We observed a larger causal effect of adiposity on fasting insulin levels in men than in women when using the *FTO* instrument. We found that several estimates from the observational analyses were significantly different from the causal estimates in the old stratum.

Causal Effects of Adiposity on Cardiovascular Risk Factors

We confirm previous evidence that adiposity causally affects a broad range of cardiovascular risk factors. Adipose tissue has been shown to secrete >50 hormones

and signaling molecules, many of them being proinflammatory or anti-inflammatory, and thereby exerting effects on insulin sensitivity (25). Adipose cells from obese individuals have higher secretion of proinflammatory cytokines such as tumor necrosis factor-α and IL-6 than cells from lean individuals (26), as well as increased secretion of adiponectin, which has been shown to increase insulin sensitivity (27). Decreased insulin sensitivity (i.e., insulin resistance) has been suggested to be a major link between obesity and cardiovascular risk factors such as hypertension, dyslipidemia, nonalcoholic fatty liver disease, and type 2 diabetes (28–30). Nevertheless, the metabolic consequences of obesity are complex. For example, nonalcoholic fatty liver disease is known to increase insulin resistance regardless of obesity, and thereby to increase the risk of diabetes. The results of the current study do not elucidate the precise mechanisms of how adiposity leads to the various metabolic phenotypes but, rather, clarify and quantify the causal role of adiposity and delineate its role in different age and sex groups.

Sex Differences in the Effects of Adiposity on Cardiovascular Risk Factors

Men and women show a sexual dimorphism in body fat proportion and distribution, and for a given BMI, women have more adipose tissue than men. However, men are more likely to deposit visceral fat in the abdominal region, while women tend to deposit their fat subcutaneously and in their lower extremities. Excess adipose tissue in the abdominal region, especially visceral fat, is associated with many of the cardiovascular risk factors (31). We found a stronger association of BMI with blood pressure, insulin, liver markers, and circulating lipids in men than in

Table 3—Age-stratified observational and IV analysis based on a genetic score of the association of adiposity with cardiovascular risk factors

		<55 years				≥55 years			
	n	β (95% CI)	P*	P_IV-obs*	n	β (95% CI)	P*	P_IV-obs*	P_diff*
Observational analyses									
Diastolic blood pressure	71,588	0.29 (0.26–0.32)	2.7×10^{-98}		48,210	0.23 (0.19–0.27)	4.4×10^{-35}		0.01
Systolic blood pressure	71,753	0.25 (0.22–0.27)	2.3×10^{-85}		48,596	0.22 (0.18–0.26)	2.0×10^{-26}		0.19
Fasting glucose	45,047	0.16 (0.13–0.20)	7.3×10^{-21}		25,089	0.24 (0.20–0.27)	4.7×10^{-37}		0.005
2-h post-OGTT glucose	13,778	0.18 (0.10–0.25)	3.1×10^{-6}		8,426	0.21 (0.09–0.32)	4.6×10^{-4}		0.66
HbA1c	14,824	0.13 (0.07–0.20)	1.1×10^{-4}		14,397	0.18 (0.12–0.24)	8.8×10^{-9}		0.35
ln fasting insulin	26,322	0.48 (0.44–0.52)	1.8×10^{-118}		14,815	0.50 (0.45–0.54)	1.6×10^{-97}		0.57
ln CRP	41,854	0.40 (0.37–0.43)	2.5×10^{-175}		30,090	0.30 (0.26–0.33)	2.4×10^{-68}		2.51×10^{-6}
ln IL-6	6,719	0.16 (0.06–0.26)	0.001		5,159	0.14 (0.08–0.21)	1.9×10^{-5}		0.73
HDL-C	57,672	−0.28 (−0.30 to −0.27)	1.1×10^{-304}		36,171	−0.28 (−0.31 to −0.25)	9.1×10^{-99}		0.83
LDL-C	56,392	0.16 (0.13–0.18)	4.4×10^{-33}		31,499	0.02 (0.00–0.04)	0.08		9.73×10^{-16}
Total cholesterol	69,736	0.12 (0.10–0.15)	9.8×10^{-23}		39,112	0.00 (−0.02 to 0.02)	0.96		1.0×10^{-12}
ln triglycerides	68,777	0.33 (0.31–0.35)	4.3×10^{-224}		34,061	0.27 (0.25–0.29)	9.4×10^{-181}		2.4×10^{-5}
ln ALT	27,956	0.24 (0.18–0.30)	1.1×10^{-13}		17,517	0.19 (0.17–0.21)	3.1×10^{-69}		0.2
ln GGT	47,202	0.25 (0.22–0.28)	4.5×10^{-50}		24,657	0.18 (0.16–0.20)	4.5×10^{-65}		5.5×10^{-4}
IV analyses									
Diastolic blood pressure	35,518	0.21 (0.09–0.33)	8.0×10^{-4}	0.20	32,596	0.01 (−0.16 to 0.18)	0.93	0.01	0.06
Systolic blood pressure	35,681	0.21 (0.12–0.30)	2.5×10^{-6}	0.41	32,989	0.06 (−0.14 to 0.26)	0.56	0.13	0.17
Fasting glucose	20,742	0.10 (−0.01 to 0.22)	0.09	0.29	17,380	0.04 (−0.11 to 0.19)	0.58	0.01	0.54
2-h post-OGTT glucose	2,077	−0.01 (−0.41 to 0.39)	0.97	0.38	2,519	0.13 (−0.27 to 0.53)	0.53	0.71	0.64
HbA1c	13,614	0.10 (−0.05 to 0.24)	0.19	0.65	13,287	0.25 (0.01–0.48)	0.04	0.58	0.29
ln fasting insulin	15,985	0.26 (0.10–0.42)	0.001	0.01	9,526	0.32 (0.12–0.52)	0.002	0.09	0.64
ln CRP	25,822	0.35 (0.23–0.48)	7.5×10^{-8}	0.48	21,943	0.18 (0.01–0.35)	0.04	0.17	0.10
ln IL-6	5,368	0.37 (0.12–0.61)	0.004	0.13	4,917	0.13 (−0.13 to 0.40)	0.32	0.96	0.21
HDL-C	32,977	−0.36 (−0.47 to −0.25)	3.4×10^{-10}	0.20	26,425	−0.28 (−0.40 to −0.17)	1.2×10^{-6}	0.97	0.36
LDL-C	32,016	0.15 (0.05–0.25)	0.004	0.92	21,851	−0.10 (−0.32 to 0.12)	0.38	0.30	0.04
Total cholesterol	33,002	0.10 (−0.05 to 0.24)	0.20	0.73	27,344	−0.19 (−0.36 to −0.01)	0.04	0.04	0.015
ln triglycerides	33,018	0.28 (0.16–0.41)	1.3×10^{-5}	0.45	22,438	0.12 (0.00–0.25)	0.05	0.02	0.09
ln ALT	11,215	0.22 (0.05–0.39)	0.01	0.84	12,712	−0.10 (−0.39 to 0.19)	0.50	0.05	0.06
ln GGT	13,915	0.24 (0.10–0.38)	0.001	0.87	12,916	−0.01 (−0.24 to 0.21)	0.92	0.10	0.07

All models were adjusted for sex and age. β (95% CI), effect per SD change of BMI on trait (SD scale); P_diff, difference between the two strata (only significant P values are shown); P_IV-obs, P value from test of difference between observational and IV analyses. *A nominal P value of <0.022 is considered significant at a 5% FDR level.

Table 4—Sex-stratified observational and IV analysis based on a genetic score of the association of adiposity with cardiovascular risk factors

	Women				Men				
	n	β (95%CI)	P*	P_IV-obs*	n	β (95% CI)	P*	P_IV-obs*	P_diff*
Observational analyses									
Diastolic blood pressure	59,738	0.22 (0.20–0.24)	2.5×10^{-101}		57,492	0.29 (0.27–0.32)	8.8×10^{-106}		3.3×10^{-5}
Systolic blood pressure	59,859	0.21 (0.18–0.23)	3.4×10^{-77}		57,922	0.24 (0.22–0.27)	1.76×10^{-94}		0.01
Fasting glucose	35,823	0.18 (0.15–0.21)	2.9×10^{-26}		33,288	0.20 (0.15–0.24)	1.2×10^{-17}		0.48
2-h post-OGTT glucose	10,248	0.16 (0.05–0.28)	0.01		11,956	0.22 (0.15–0.28)	9.51×10^{-11}		0.41
HbA$_{1c}$	15,676	0.17 (0.12–0.21)	9.1×10^{-14}		13,775	0.18 (0.09–0.27)	5.6×10^{-5}		0.76
ln fasting insulin	22,137	0.42 (0.38–0.46)	7.6×10^{-98}		18,027	0.58 (0.52–0.65)	1.1×10^{-73}		2.0×10^{-5}
ln CRP	40,072	0.37 (0.34–0.40)	5.0×10^{-107}		31,888	0.30 (0.27–0.33)	9.3×10^{-71}		0.002
ln IL-6	6,652	0.15 (0.08–0.22)	8.0×10^{-5}		5,226	0.10 (0.05–0.16)	2.0×10^{-4}		0.31
HDL-C	49,949	−0.27 (−0.30 to −0.25)	5.2×10^{-150}		43,039	−0.29 (−0.31 to −0.27)	1.4×10^{-194}		0.36
LDL-C	47,619	0.11 (0.08–0.13)	2.5×10^{-20}		39,174	0.11 (0.07–0.15)	1.8×10^{-8}		0.73
Total cholesterol	55,223	0.06 (0.04–0.08)	3.6×10^{-10}		52,518	0.11 (0.08–0.14)	1.5×10^{-13}		2.6×10^{-16}
ln triglycerides	52,873	0.26 (0.24–0.28)	4.5×10^{-182}		48,836	0.39 (0.36–0.41)	4.2×10^{-204}		0.005
ln ALT	24,013	0.16 (0.13–0.19)	1.9×10^{-28}		21,460	0.32 (0.28–0.36)	1.6×10^{-55}		5.9×10^{-11}
ln GGT	38,127	0.17 (0.15–0.20)	2.3×10^{-48}		33,732	0.31 (0.28–0.33)	2.2×10^{-96}		2.16×10^{-12}
IV analyses									
Diastolic blood pressure	34,333	0.07 (−0.04 to 0.18)	0.24	0.01	32,664	0.23 (0.06–0.40)	0.008	0.44	0.11
Systolic blood pressure	34,459	0.10 (−0.01 to 0.20)	0.06	0.05	33,094	0.21 (0.04–0.37)	0.02	0.65	0.29
Fasting glucose	22,066	0.09 (−0.03 to 0.21)	0.14	0.15	15,104	0.10 (−0.06 to 0.26)	0.23	0.24	0.93
2-h post-OGTT glucose	2,360	0.18 (−0.22 to 0.57)	0.39	0.95	2,225	0.08 (−0.55 to 0.72)	0.80	0.68	0.81
HbA$_{1c}$	14,332	0.13 (−0.01 to 0.28)	0.06	0.69	12,569	0.28 (0.06–0.51)	0.01	0.41	0.28
ln fasting insulin	14,085	0.29 (0.14–0.45)	3.0×10^{-4}	0.12	10,518	0.36 (0.02–0.70)	0.04	0.19	0.73
ln CRP	27,107	0.29 (0.09–0.49)	0.004	0.45	20,674	0.26 (0.12–0.40)	3.0×10^{-4}	0.59	0.79
ln IL-6	5,840	0.38 (0.14–0.62)	0.002	0.07	4,445	0.08 (−0.22 to 0.37)	0.62	0.86	0.11
HDL-C	31,201	−0.34 (−0.45 to −0.23)	7.9×10^{-10}	0.22	27,146	−0.31 (−0.46 to −0.16)	4.0×10^{-5}	0.75	0.74
LDL-C	29,250	0.10 (−0.01 to 0.22)	0.08	0.96	23,595	0.04 (−0.09 to 0.17)	0.60	0.26	0.45
Total cholesterol	31,515	−0.02 (−0.14 to 0.09)	0.68	0.96	27,796	0.03 (−0.13 to 0.18)	0.74	0.11	0.88
ln triglycerides	29,744	0.25 (0.14–0.37)	2.8×10^{-5}	0.16	24,660	0.24 (0.06–0.42)	0.01	0.30	0.61
ln ALT	14,701	0.11 (−0.04 to 0.26)	0.14	0.51	9,226	0.09 (−0.14 to 0.32)	0.43	0.05	0.90
ln GGT	16,794	0.13 (−0.06 to 0.32)	0.18	0.64	10,037	0.17 (−0.09 to 0.43)	0.20	0.32	0.80

All models were adjusted for age. P_{diff}, difference between men and women (only significant P values are shown); $P_{IV\text{-}obs}$, P value from test of difference between observational and IV analyses; β (95% CI), effect per SD change of BMI on trait (SD scale); ln, transformed to natural logarithm scale. *A nominal P value of <0.022 is considered significant at a 5% FDR level.

women in the observational analyses. Also, our results from the causal analyses using the *FTO* as an instrument suggest that a given increase in overall adiposity causes larger detrimental effects on insulin sensitivity in men than in women. This observation is consistent with previous observational studies and extends these results by demonstrating the difference in causal (IV) estimates (12,32). Moreover, in the observational analyses, we observed larger effect estimates of adiposity on CRP in women than in men, which is consistent with previous findings (33). It has been speculated that this sex heterogeneity could originate from differences in adipose endocrine function (34–36).

Age Differences in the Effects of Adiposity on Cardiovascular Risk Factors

The proportion of visceral adipose tissue generally increases with age, particularly among men and postmenopausal women, leading to increased insulin resistance (11,37). Weight loss in older people has been shown to reduce inflammatory markers but also to reduce muscle mass and bone mass density. This has led to a debate about whether weight loss interventions should be recommended at all to elderly obese individuals (38). Because of this apparent differential effect of weight changes in older and younger people, we carried out the analyses after stratification of participants according to age (<55 or ≥55 years). We chose the age cutoff of 55 years for the following reasons: 1) to achieve comparable numbers of studies with information available for the two age strata and 2) most women have entered menopause by 55 years of age. For power reasons, we could not stratify on more than two age groups. We found associations between adiposity and markers of inflammation, liver damage, blood pressure, and lipid concentrations to be attenuated by age and only the association with fasting glucose to be augmented. Differences in causal point estimates between the two age strata were more extreme than the differences in observational estimates, with an almost abolished effect of adiposity on several traits such as blood pressure in the ≥55-year stratum using IV analysis, although formal statistical tests for differences were, in general, nonsignificant (likely due to the inherent lower precision of causal than observational estimates). Our genetic score instrument was stronger in the <55-year age group than in the ≥55-year age group, as can be seen from a fourfold difference in *F* statistic values. This difference yielded slightly larger SEs for the IV estimates in the ≥55-year-old group, but should not bias the point estimates. The observed age-declining effects of adiposity on nonglycemic traits, such as blood pressure and circulating lipid levels, are in line with the limited prior literature (13,15). We speculate that these results could be caused by age-related changes in vascular biology and lipid metabolism, or could be explained by survival bias or a greater prescription of lipid-lowering therapy in older obese individuals. Further, we found that several

of the estimates derived from cross-sectional observational analysis were significantly larger than IV estimates in the ≥55-year stratum. We believe that the IV estimates are better estimates of the causal effect of adiposity on cardiovascular risk factors, and that observational estimates in this age group should be interpreted with caution. We speculate that the observed error in the observational analysis is caused by confounders, such as the presence of other diseases or subclinical CVD, not accounted for in the models that can affect both BMI and CVD risk factors.

Strengths and Limitations

The main strengths of the present investigation include the large study size, allowing for age and sex stratification and the examination of a wide range of cardiovascular risk factors. The limitations are mainly related to the validity of the assumptions underlying causal interpretation within Mendelian randomization studies, as follows: 1) independence between the instrument and confounders (i.e., genotypes are randomized); 2) a reliable association between the genetic variant and intermediate phenotype; and 3) conditional independence between the genetic variant and the outcome, given the intermediate phenotype and the confounders (i.e., lack of pleiotropy) (9).

Possible violations of the first and the third assumptions include population stratification, pleiotropic effects, canalization, epigenetic effects, and the presence of genes associated with confounders and outcomes in LD with the genetic variants used. Neither the first nor the third assumption can be tested statistically, and conclusions about the validity of these assumptions in a given study have to be based on previous biological knowledge. In the current study, all association analyses were performed within each study separately (including individuals from a similar genetic background), and all studies included only individuals of European ancestry. Hence, bias from population stratification is deemed unlikely. With regard to the possibility of pleiotropic effects by genes included in the genetic score, we acknowledge that for most loci, neither the causal genes nor their precise effects are known. A combined genetic score, such as the one used in our study, has been suggested to balance the possible pleiotropic effects of some included SNPs (39), but there is controversy about this notion (40). Some of the SNPs with the largest effect on BMI, such as for variants in the close vicinity to *FTO* and *MC4R*, also come up in genome-wide association studies for other traits, such as HDL-C. The effects on non-BMI traits are usually proportional to their effects on BMI itself, which is a sign of mediation rather than a sign of these SNPs being pleiotropic (41). Finally, we found similar results when using the genetic score compared with those using the single SNP instrument, which argues against strong pleiotropic effects as the explanation of our results.

Another limitation is that, despite the very large sample size and strong associations of the instruments with BMI, our analysis still obtained results with wide CIs for the IV

estimators, highlighting modest power. The sample sizes in IV analyses varied with phenotype and strata from 2,077 individuals (age <55 years, 2-h post-OGTT glucose levels) to 67,553 individuals (nonstratified analysis, systolic blood pressure), yielding larger SEs in the smaller groups, and lower statistical power to detect causal effects and differences between strata. This should be taken into consideration when interpreting the results. Based on a pilot study (data not shown), we chose to use a nonweighted genetic score. The use of such a nonweighted score compared with a weighted score in IV analysis has been evaluated by Burgess and Thompson (40) under different settings, and was found to yield less power but unbiased IV estimates. They further conclude that mis-specifications of the genetic model, such as nonlinear genetic effects, or effect modifications by variant-variant or variant-environment interactions do not lead to significant bias.

Conclusion

For the first time in a large Mendelian randomization study, we applied stratified analysis to assess differences in the causal effect of adiposity on cardiovascular risk factors across age and sex groups. For total cholesterol levels, causal and observational estimates were larger in the <55-year stratum than in the ≥55-year stratum, a finding that could be explained by biology, survival bias, or differential medication in older obese subjects.

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