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Lifetime body mass index and later atherosclerosis risk in young adults: examining causal links using Mendelian randomization in the Cardiovascular Risk in Young Finns study

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Aims Mendelian randomization uses genetic variants related to environmentally modifiable risk factors in an attempt to improve causal inference from observational data. We examined the effect of lifetime body mass index (BMI) on adult carotid intima-media thickness (CIMT) and various atherosclerotic risk factors by using both Mendelian randomization and conventional analyses.

Methods and results A total of 2230 individuals (1218 women), aged 3–18 at study induction, took part in clinical examinations in 1980, 1983, 1986, and, most recently, 2001 when they were aged 24–39. In these analyses we utilized the known relation between *FTO* polymorphism rs9939609 and BMI. The dose–response association between the number of A alleles in *FTO* and higher mean BMI from childhood to adulthood was confirmed, but no associations with potential confounding factors were observed. In standard regression models, lifetime BMI was associated with adult CIMT, lifetime systolic blood pressure, adult fasting glucose, and adult HOMA-index. When variation in *FTO* was used as an instrument for unconfounded BMI levels, similar or larger effects of lifetime BMI on all these phenotypes were found, although with wider confidence intervals.

Conclusion Mutually supportive results from Mendelian randomization and standard regression models strengthen the evidence of the effect of lifetime BMI on atherosclerosis risk in young adults.

Keywords Atherosclerosis • Body mass index • Mendelian randomization • Variation (genetics)

Introduction

The limitations of observational cohort studies to determine causal associations between hypothesized risk factors and important chronic diseases, such as coronary heart disease (CHD), are well documented.[1] First, because many potential risk factors are associated with a range of other predictor variables, observed associations may be because of confounding. Secondly, because

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CHD develops over the lifecourse,[2] reverse causality, where early stages of atherosclerosis and/or preclinical heart disease affect risk factor levels, rather than *vice versa*, also remains a distinct possibility.

Mendelian randomization has recently been advanced as a means of dealing with both confounding and reverse causality. This approach is predicated upon the random assortment of alleles at the time of gamete formation that leads to population distributions of genetic variants that are generally independent of the environmental exposures that commonly confound epidemiological risk factors–disease associations.[3–6] These unconfounded differences in risk factor levels should therefore translate into differences in disease occurrence if the exposure is truly causally related to the disease. Moreover, genetic variants will not be influenced by the existing cardiovascular pathology and therefore reverse causation will not distort the association between genotype and disease in the way it would distort the relationship between risk factor levels measured in adulthood and disease. Finally, genetic variants typically affect relevant protein levels throughout life, so reducing the problem of attenuation of effects (regression dilution bias) common in cohort studies with extended follow-up.

Data from the Cardiovascular Risk in Young Finns study[7] enabled us to examine the associations of lifetime body mass index (BMI, kg/m^2) with adult carotid intima-media thickness (CIMT), a valid preclinical marker of CHD, and atherosclerotic risk factors, such as blood pressure, lipids, and C-reactive protein, across the life course from age 3–39 years in a racially homogenous white European population. We utilized the known association of variants in the *FTO* gene with BMI[8–11] to examine whether the Mendelian randomization approach would provide support for a causal association between lifetime BMI and atherosclerosis risk (*Figure 1*). Thus, individuals with A alleles in the *FTO* polymorphism rs9939609 are predisposed to higher adiposity[8] and they have in effect been randomly allocated to somewhat higher levels of BMI than individuals with other genotypes related to lower adiposity (paths a and b). Eventually, these differences in BMI should confer differences in atherosclerosis and atherosclerotic risk (path c) factors if BMI was causally related to them and population stratification did not confound the associations.[12] To our knowledge, this is the first Mendelian randomization study to examine the effect of lifetime BMI on atherosclerosis risk based on direct structural vascular measurement.

Methods

Study population

The Young Finns study is a multicentre follow-up study of cardiovascular risk factors in Finnish children and adolescents.[7,13] The original sample was 4320 Caucasian children and adolescents aged 3, 6, 9, 12, 15, and 18 years who were randomly sampled from five areas of Finland using the national register. The baseline examination was conducted in 1980, with response being 83% (3596 of those invited). Follow-ups were conducted in 1983, 1986, and, the latest, 2001 when the participants had reached 24–39 years of age. The 2230 individuals (1012 men and 1218 women) with full data on *FTO* polymorphism rs9939609 and BMI in childhood and adulthood formed the present analytical sample (*Figure 2*). Differences in the baseline characteristics of participants and non-participants were small but achieved statistical significance owing to the large numbers: BMI (17.9 vs. 17.8 kg/m^2, $P = 0.22$); age (10.7 vs. 10.0 years, $P < 0.0001$); and sex (55% vs. 45% female, $P < 0.0001$). The study was conducted according to the guidelines of the Helsinki declaration, and the study protocol was approved by local ethics committees. All participants gave their full informed consent.

FTO genotyping

Genomic DNA was extracted from peripheral blood leucocytes using a commercially available kit and Qiagen BioRobot M48 Workstation according to the manufacturer's instructions (Qiagen Inc., Hilden, Germany). We genotyped *FTO* single nucleotide polymorphism (SNP) rs9939609 by employing the 5′ nuclease assay and fluorogenic allele-specific TaqMan MGB probes,[14] using the ABI Prism 7900HT Sequence Detection System (Applied Biosystems, Foster City, CA, USA). The samples were pipetted using an automated TECAN Freedom

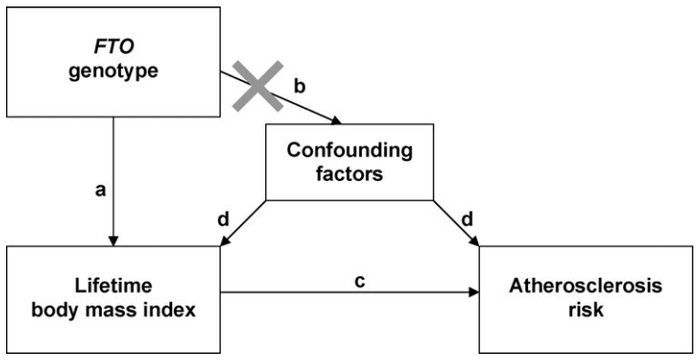

Figure 1 The Mendelian randomization using instrumental variables regression analysis examines whether lifetime BMI, to the extent it is associated with *FTO* genotype (*path a*), predicts atherosclerosis risk (*path c*). *FTO* genotype is expected to be unrelated to confounding factors (*path b*) and thus the effects of confounding factors (*path d*) are expected to be eliminated in the analysis. Conventional regression analysis, in contrast, is open to bias because of unmeasured or imprecisely measured confounding factors.

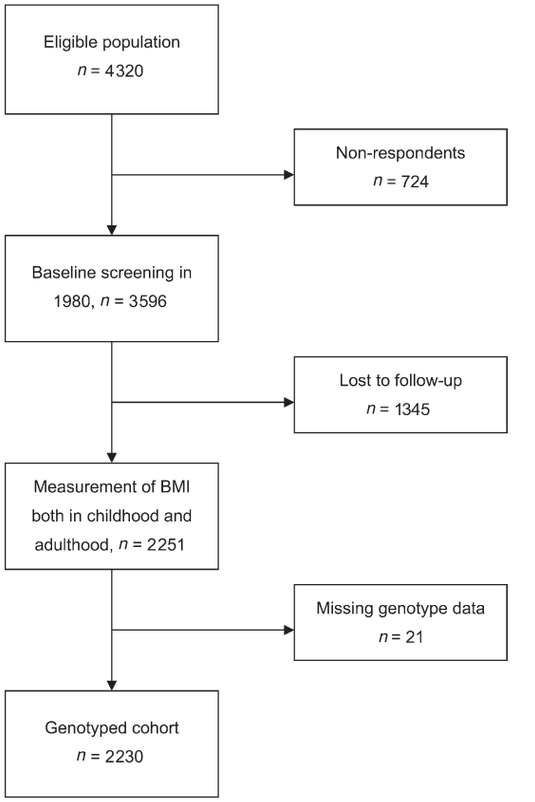

Figure 2 Sample selection.

EVO-100 instrument (Tecan Group Ltd, Männedorf, Switzerland). The nucleotide sequences of the primers and probes used in the polymerase chain reaction (PCR) were deduced from published sequences deposited in the GenBank and Celera databases and synthesized by Applied Biosystems. The PCR containing genomic DNA, 1× Universal PCR Master Mix, 900 nM of each primer and 200 nM of each probe were performed in 384-well plates using the standard protocol in a total volume of 5 μL. Endpoint fluorescence was measured post-PCR and genotype calling carried out by the allelic discrimination analysis module (ABI Prism SDS software, ABI, Foster City, CA, USA). Random duplicates were used as quality control. All genotypes were analysed in the Department of Clinical Chemistry at Tampere University Hospital and University of Tampere.

Body mass index and other life course atherosclerotic risk factors

Life course measures were based on repeated assessments in 1980 (participant age range 3–18 years), 1983 (6–21 years), 1986 (9–24 years), and 2001 (24–39 years), except C-reactive protein, which was assessed in 1980 and 2001 only. Measurements of weight (kg) and height (mm) were obtained using standard research protocols and were used to calculate BMI (weight in kg/height^2 in m).[15] Measurements of blood pressure were taken with the participant in the sitting position after 5 min rest with a mercury sphygmomanometer (for the 3 year olds with ultrasound device, Arteriosonde 1020, Roche) in 1980, 1983, and 1986 and with a random zero sphygmomanometer (Hawksley & Sons Ltd, West Sussex, UK) in 2001.[16,17] Readings to the nearest even number of millimetres of mercury were performed at least three times on each subject; the average of the systolic blood pressure measurements was used in the analysis.

In 1980, 1983, 1986, and 2001, all blood samples were taken after an overnight fast and analysed in duplicate in the same laboratory on each time occasion.[7] Standard enzymatic methods were used for serum total cholesterol, HDL-cholesterol and triglycerides, and plasma glucose concentrations. LDL-cholesterol concentration was calculated using the Friedewald formula.[18] In 2001, we assessed serum high-sensitive C-reactive protein using an automated analyser (Olympus AU400, Olympus, USA) and a highly sensitive turbidimetric immunoassay kit (CRP-UL-assay, Wako Chemicals, Neuss, Germany). Serum C-reactive protein samples taken in 1980 were stored at −20°C and analysed in 2005 using the same method as in 2001. During the storage, the samples were not thawed or refrozen.

Carotid intima-media thickness and adulthood atherosclerotic risk factors

In 2001–2002, ultrasound studies were performed using Sequoia 512 ultrasound mainframes (Acuson, Mountain View, CA, USA) to measure CIMT. The measurement has previously been described in detail.[7,19] In brief, the image was focused on the posterior (far) wall of the left carotid artery. A minimum of four measurements of the common carotid far wall were taken approximately 10 mm proximal to the carotid bifurcation to derive mean carotid IMT. The digitally stored scans were manually analysed by one reader blinded to subjects' details (M.J.). The between-visit coefficient of variation of IMT measurements was 6.4%.

Several additional assessments were carried out in 2001.[20] Waist circumference (mm, measured in duplicate at the level of the twelfth rib or level with the navel in thin subjects) and hip circumference (mm) were obtained to calculate waist–hip ratio. Standard enzymatic methods were used for plasma glucose concentrations. Serum insulin was measured by microparticle enzyme immunoassay kit (Abbott Laboratories, Diagnostic Division, Dainabot). Insulin resistance was estimated according to the homeostasis model as the product of fasting glucose and insulin divided by the constant 22.5, i.e. the HOMA index.[21] Serum apolipoproteins A-1 and B were analysed immunoturbidometrically (Orion Diagnostica, Espoo, Finland).

Parental factors and adult lifestyle factors

Parental socioeconomic position was measured in 1980 using parental occupational status as classified by Statistics Finland and categorized as manual vs. non-manual.[22] Where socioeconomic position differed between parents, data on the parent with the higher occupational status were used. Parental BMI in 1980 was calculated from self-reported height and weight.

The participant's own adult socioeconomic position was measured in 2001 by occupational status and categorized as for parental socioeconomic position. Educational attainment was categorized as academic; secondary education but not academic; and comprehensive school as the highest level of education. Information on adult smoking (status and pack-years of tobacco smoking) and alcohol consumption (units per week) was obtained by questionnaire in 2001. One unit of alcohol (12 g) was equal to a glass of wine, a single 4 cL measure of spirits or a 33 cL bottle of beer.[20]

Statistical analysis

Statistical significance in all tests was inferred at a two-tailed $P < 0.05$. The following tests were performed.

Preliminary genetic analyses

We tested the Hardy–Weinberg equilibrium at *FTO* SNP locus, on a contingency table of observed vs. predicted genotypic frequencies, with an exact test to evaluate whether genotyping error, biological selection bias, or other population non-homogeneity was likely in our data. The genotype frequencies in the *FTO* polymorphism were in Hardy–Weinberg equilibrium ($P > 0.20$), thus supporting the integrity of our genetic data.

Test of genotype–phenotype associations

We constructed lifetime phenotype measures for BMI, height, systolic blood pressure, total cholesterol, HDL- and LDL-cholesterol, and C-reactive protein by calculating age-standardized *z*-scores (mean = 0, standard deviation = 1) for each age and for men and women separately and then averaged across ages within each measure and participant. We used the logarithm of C-reactive protein to correct for its positively skewed distribution. There was no strong evidence of *FTO* polymorphism × sex interaction on any of the phenotype measures (all $P > 0.11$), thus the main analysis was conducted for men and women in combination. We used linear regression analysis to study the age- and sex-adjusted associations of the *FTO* genotype (per A allele) with phenotype measures. Since our analyses are all hypothesis driven and hence equivalent to a candidate gene approach, we used conventional alpha levels to detect statistically significant associations without adjusting for multiple testing. These analyses were performed with Statistical Analysis System (SAS, version 9.1).

Instrumental variables regression analysis

As in previous studies using the Mendelian randomization approach,[23–31] we performed an instrumental variables regression analysis to examine if the *FTO* polymorphism was associated with CIMT and atherosclerotic risk factors through its association with BMI levels. We compared the results from the instrumental variable estimates of the association between lifetime BMI and phenotype measures (i.e. path c in *Figure 1*) to those from standard linear regression using the Durbin form of the Durbin–Wu–Hausman statistic.[32] We used the *F*-statistics from the first-stage regressions to evaluate the strength of the instruments, i.e. path a in *Figure 1* (values >10 are taken to indicate sufficient strength to ensure the validity of instrumental variable methods).[33,34] Instrumental variable regression analysis was performed with Stata (version 9.2, Stata Institute, TX, USA).

An additional analysis with a simulated data set was carried out to examine the change in precision of the results from the instrumental variable regression analyses if our sample size was substantially larger. First, we filled the missing values in the baseline sample ($n = 3596$) by multivariate imputation method of ICE in Stata software, with missing-at-random assumptions [$n = 1410$ (39%) had some missing data]. In addition to all the variables in the analyses, the imputation process included baseline predictors of dropout from the cohort, such as BMI (available from all but one baseline participant). We imputed two independent copies of the complete baseline data and treated them as one artificially created simulated data set with a sample size of 7192. Both standard and instrumental variables regression analyses were then run with this large simulated data set. To examine whether the obtained estimates were robust, we repeated this procedure five times, thus creating five independent simulated data sets, and compared estimates between the simulations.

Results

Characteristics of the study population are shown in *Table 1*. Variation in *FTO* (rs9939609) was not associated with potential confounding factors, such as age, sex, socioeconomic circumstances, education, or risk behaviours (*Table 2*) supporting the assumption that the variation in the *FTO* genotype is independent of potential confounding factors (path b in *Figure 1*). In contrast, lower parental socioeconomic position and participant's lower educational attainment were associated with higher lifetime BMI ($P < 0.05$), demonstrating that observed BMI is affected by potential confounding factors (path d in *Figure 1*).

Gene-phenotype associations

FTO polymorphism explained 0.4% of the variation in lifetime BMI. Each additional copy of the A allele in the *FTO* gene was associated with higher lifetime BMI, with higher maternal BMI and, more weakly, paternal BMI (*Table 3*). *FTO* polymorphism was also associated with higher lifetime systolic blood pressure, but no association was found with lipids or C-reactive protein. The association between *FTO* polymorphism and lifetime systolic blood pressure attenuated after adjustment for lifetime BMI (adjusted change per A allele, 0.05; 95% CI, −0.01, 0.10; $P = 0.11$), suggesting that adiposity as indexed by BMI at least partially mediates this association.

Of the adulthood outcomes, a greater number of A alleles was associated with higher BMI (although less strongly than with lifetime BMI), larger waist circumference, and higher fasting glucose levels. Adjustment for BMI and waist circumference had little effect on the association between *FTO* polymorphism and fasting glucose (adjusted change per A allele 0.05; 95% CI, 0.01, 0.10; $P = 0.03$). A greater number of A alleles was weakly associated with greater CIMT and higher insulin resistance. Progression of atherosclerosis is more rapid in men than in pre-menopausal women. In sex-specific analysis, each additional copy of A allele was associated with a CIMT increase of 0.009 mm (95% CI, 0.000, 0.017; $P = 0.049$) in men. This figure was 0.000 mm (95% CI, −0.006, 0.007; $P = 0.95$) in women (*P* for sex interaction = 0.11; for all other genotype associations, *P* for sex interaction exceeded 0.2). Adjustment for BMI and waist circumference slightly reduced the effect of *FTO* genotype on CIMT in men (per A allele change, 0.008; 95% CI, −0.001, 0.016; $P = 0.08$).

Standard regression analysis and instrumental variables regression analysis

The standard regression analysis suggests positive associations of lifetime BMI with adult CIMT, lifetime systolic blood pressure, adult glucose, and adult insulin resistance (*Table 4*, upper panel). In the instrumental variables regression analysis with *FTO* polymorphism as the instrument for unconfounded lifetime BMI levels (path a in *Figure 1*), all the effects of BMI on CIMT and other phenotypes are in the same direction but less precisely estimated (*P*-values range between 0.02 and 0.15). In general, the effects appeared larger in the instrumental analysis than in the standard regression analysis, but only for adult fasting glucose does the difference in effect sizes between the two analyses reach conventional statistical significance. The wide confidence

Table 1 Characteristics of study sample

	Female		Male	
	n	Mean (SD) or %	*n*	Mean (SD) or %
Mean age at baseline (year)	1218	10.7 (5.0)	1012	10.7 (5.0)
FTO genotype (rs9939609) (%)				
TT	420	34.5	367	36.3
TA	592	48.6	495	48.9
AA	206	16.9	150	14.8
Mother's BMI (kg/m^2)	1185	24.0 (3.8)	986	23.9 (3.7)
Father's BMI (kg/m^2)	1069	25.5 (3.1)	873	25.4 (3.1)
BMI in childhood and adolescence (kg/m^2)				
Age 3	173	15.5 (1.2)	148	15.6 (1.0)
Age 6	371	15.6 (1.7)	290	15.5 (1.7)
Age 9	562	16.6 (2.3)	465	16.6 (2.1)
Age 12	600	18.3 (2.7)	485	18.1 (2.6)
Age 15	601	20.2 (2.5)	503	20.0 (2.5)
Age 18	554	21.2 (2.6)	429	21.5 (2.7)
Adult measures (age 24–39)				
Carotid IMT (mm)	1207	0.573 (0.084)	1004	0.593 (0.100)
BMI (kg/m^2)	1218	24.5 (4.6)	1012	25.7 (4.1)
Waist–hip ratio	1218	0.79 (0.06)	1012	0.90 (0.06)
Waist circumference (mm)	1218	794 (114)	1012	898 (108)
Height (cm)	1218	166.0 (59.6)	1012	179.5 (65.4)
Systolic BP (mmHg)	1205	116.0 (12.4)	1000	129.3 (13.5)
TC (mmol/L)	1218	5.05 (0.91)	1012	5.26 (1.04)
HDL-cholesterol (mmol/L)	1218	1.39 (0.30)	1010	1.16 (0.28)
LDL-cholesterol (mmol/L)	1218	3.14 (0.77)	1010	3.42 (0.92)
TG (mmol/L)	1218	1.16 (0.67)	1012	1.54 (1.00)
Log C-reactive protein (mmol/L)	1218	−0.09 (1.82)	1012	−0.54 (1.65)
Glucose (mmol/dL)	1218	4.92 (0.74)	1012	5.23 (0.92)
Insulin (mmol/dL)	1218	7.83 (5.92)	1011	7.72 (5.80)
HOMA index	1218	1.77 (1.80)	1011	1.87 (2.01)
Apolipoprotein A-1 (g/L)	1218	1.56 (0.26)	1012	1.40 (0.21)
Apolipoprotein B (g/L)	1218	0.99 (0.24)	1012	1.14 (0.27)

intervals in the instrumental variables regression analysis suggest that our data, despite the relatively large sample size, had limited power for the instrumental variable analyses (i.e. path a in *Figure 1* was relatively weak). Indeed, *F*-values in the first-stage regression testing the strength of instruments were <10 (range, 7.5–9.2). Running these analyses with *FTO* haplotypes as instruments did not solve this problem as the strength of instrument was not improved (see Supplementary material online, Appendix 1).

The lower panel of *Table 4* shows the results from the standard and instrumental variables regression analyses using simulated data corresponding to ours, but the sample size increased two-fold to that we had at the baseline (i.e. $n = 2 \times 3596$). In such data, *F*-value in the first-stage regression is 31.6; all of the associations of lifetime BMI with CIMT and atherosclerotic risk factors were now robust in instrumental variables regression analysis and for all outcomes except HOMA index these associations were of greater magnitude compared with those in the standard regression analyses. We repeated these analyses with five independently simulated data sets. The associations in instrumental variables regression analysis and standard regression analysis remained across all of the simulations repeated five times, whereas the difference in the magnitude of the associations between the two analyses was consistently replicated only for adult glucose (see Supplementary material online, Appendix 2).

Discussion

This study took advantage of the robust association between *FTO* polymorphism rs9939609 and BMI to conduct a Mendelian randomization analysis on the effect of lifetime BMI on CIMT and various atherosclerotic risk factors in a cohort of young adults. As expected, standard regression analysis showed lifetime BMI to be associated with greater adult CIMT, higher lifetime

Table 2 Associations between *FTO* polymorphism rs9939609 and potential confounding factors

	n	% or mean (SD) by *FTO* genotype			Change[a] (95% CI)	*P* for trend[a]
		TT	AT	AA	Per A allele	
Sex (% of men)	2230	46.6	45.5	42.1	−0.02 (−0.05, 0.01)	0.19
Age (years)[b]	2230	10.6 (4.9)	10.6 (5.0)	11.0 (5.1)	0.15 (−0.15, 0.45)	0.32
Parental socioeconomic position (% of manual)[b]	2171	38.4	40.6	39.5	0.01 (−0.02, 0.04)	0.62
Own adult socioeconomic position (% of manual)[c]	1918	32.4	33.1	31.8	0.00 (−0.03, 0.03)	0.88
Education (% of academic)[c]	2218	16.8	19.9	19.0	0.001 (−0.002, 0.005)	0.21
Smoking (% of smokers)[c]	2170	24.6	24.1	25.3	0.00 (−0.02, 0.03)	0.77
Pack-years among smokers[c]	507	10.2 (7.9)	9.2 (6.5)	9.1 (7.2)	−0.34 (−1.17, 0.48)	0.41
Alcohol consumption (units per week)[c]	2199	6.3 (9.2)	5.8 (8.0)	7.0 (8.1)	−0.12 (−0.61, 0.37)	0.64

[a]Adjusted for age and sex.
[b]Participants at age 3–18.
[c]Measured at age 24–39.

Table 3 Associations between *FTO* polymorphism rs9939609 and phenotypes across the life course and in adulthood

	n	Mean (SD) by *FTO* genotype			Change[a] (95% CI)	*P* for trend[a]
		TT	AT	AA	Per A allele	
Parental phenotype						
Mother's BMI, kg/m^2	2171	23.7 (3.7)	23.9 (3.7)	24.5 (4.1)	0.36 (0.14, 0.58)[b]	0.001
Father's BMI, kg/m^2	1940	25.3 (3.1)	25.6 (3.1)	25.6 (3.0)	0.14 (−0.06, 0.34)[b]	0.16
Lifetime phenotype[c] (mean *z*-score)						
BMI	2230	−0.07 (0.99)	0.02 (1.01)	0.11 (0.98)	0.09 (0.03, 0.15)	0.003
Systolic BP	2204	−0.06 (1.02)	0.01 (0.97)	0.10 (1.03)	0.08 (0.02, 0.14)	0.01
TC	2226	−0.01 (1.00)	0.01 (0.98)	−0.05 (1.04)	−0.01 (−0.07, 0.05)	0.70
HDL-cholesterol	2224	0.01 (1.01)	−0.02 (0.99)	0.03 (1.01)	0.00 (−0.06, 0.06)	0.95
LDL-cholesterol	2224	−0.01 (0.99)	0.01 (0.99)	−0.05 (1.05)	−0.01 (−0.07, 0.05)	0.69
TG	2228	−0.02 (1.06)	0.02 (0.97)	−0.06 (0.96)	−0.01 (−0.07, 0.05)	0.85
C-reactive protein[d]	2133	−0.02 (1.02)	−0.01 (0.98)	0.07 (1.00)	0.04 (−0.02, 0.10)	0.22
Adult phenotype[e]						
CIMT, mm	2211	0.578 (0.087)	0.582 (0.095)	0.587 (0.097)	0.003 (−0.001, 0.009)	0.14
BMI, kg/m^2	2230	24.9(4.5)	25.1 (4.3)	25.4 (4.5)	0.28 (0.02, 0.54)	0.04
Waist–hip ratio	2230	0.840 (0.080)	0.841 (0.083)	0.845 (0.082)	0.003 (−0.000, 0.007)	0.07
Waist circumference, mm	2230	837 (123)	842 (122)	848 (124)	6.5 (−0.1, 13.0)	0.05
Height, cm	2230	172.1 (8.9)	172.3 (9.1)	171.6 (9.6)	0.1 (−0.3, 0.5)	0.60
Glucose, mmol/dL	2230	5.04 (0.71)	5.03 (0.65)	5.19 (1.41)	0.06 (0.01, 0.11)	0.01
Insulin, mmol/dL	2229	7.50 (4.79)	8.01 (6.58)	7.69 (5.71)	0.18 (−0.17, 0.54)	0.31
HOMA index	2229	1.73 (1.44)	1.86 (2.05)	1.88 (2.28)	0.08 (−0.03, 0.20)	0.13
Apolipoprotein A-1, g/L	2230	1.50 (0.25)	1.48 (0.24)	1.50 (0.27)	−0.01 (−0.02, 0.01)	0.43
Apolipoprotein B, g/L	2230	1.06 (0.27)	1.06 (0.26)	1.04 (0.27)	−0.01 (−0.02, 0.01)	0.35

Note P for all interactions with sex ≥ 0.11.
[a]Adjusted for age and sex.
[b]Analysis is additionally adjusted for parental age.
[c]Measured at ages 3, 6, 9, 12, 15, 18, 24, 27, 30, 33, 36, and 39 years. Only those participants with data measured in adulthood (age 24–39) and at least once between ages 3–18 included in the analysis.
[d]Standardized after logarithmic transformation.
[e]Measured at age 24–39.

systolic blood pressure, higher adult fasting glucose, and higher HOMA index. When *FTO* polymorphism was used as an instrument for non-confounded BMI levels, similar or larger effects of lifetime BMI on all these phenotypes were found, but for statistically robust findings our sample size would need to be considerably larger.

Table 4 Comparison of the association of lifetime BMI with carotid intima-media thickness (CIMT), blood pressure, glucose, and insulin resistance obtained from ordinary least squares linear regression to that obtained from the instrumental variables regression analysis[a]

Outcome	*n*	Beta (95% CI) for outcome per 1 SD increase in sex- and age-standardized lifetime BMI z-score		*P* for difference in effect size between the two analytical approaches
		Ordinary least squares linear regression	Instrumental variables analysis[a]	
Study population				
Adult CIMT, mm	2211	0.011 (0.007 to 0.015) $P < 0.0001$	0.048 (−0.016 to 0.112) $P = 0.15$	0.23
Lifetime systolic BP, z-score	2204	0.36 (0.32 to 0.40) $P < 0.0001$	0.95 (0.15 to 1.74) $P = 0.02$	0.09
Adult glucose, mmol/dL	2230	0.08 (0.05 to 0.12) $P < 0.0001$	0.67 (−0.02 to 1.35) $P = 0.06$	0.04
Adult HOMA index	2229	0.47 (0.40 to 0.55) $P < 0.0001$	0.94 (−0.34 to 2.22) $P = 0.15$	0.46
Simulated data[b]				
Adult CIMT, mm	7192	0.013 (0.011 to 0.016) $P < 0.0001$	0.061 (0.020 to 0.101) $P = 0.003$	0.01
Lifetime systolic BP, z-score	7192	0.31 (0.29 to 0.33) $P < 0.0001$	0.60 (0.30 to 0.89) $P < 0.0001$	0.04
Adult glucose, mmol/dL	7192	0.09 (0.07 to 0.11) $P < 0.0001$	0.72 (0.32 to 1.12) $P < 0.0001$	0.0002
Adult HOMA index	7192	0.55 (0.51 to 0.60) $P < 0.0001$	0.89 (0.14 to 1.64) $P = 0.02$	0.37

[a]In instrumental variables regression analysis *FTO* polymorphism rs9939609 acts as an instrument for the unconfounded lifetime effect of BMI on CIMT and risk factors.
[b]*Post hoc* analysis with artificially generated larger sample (sample size equals two times baseline cohort).

Utility of the Mendelian randomization approach

At least three issues suggest that Mendelian randomization approach may add to the evidence from conventional cohort studies in testing the contribution of lifetime BMI to atherosclerosis risk. First, confirming previous studies, *FTO* polymorphism was associated with BMI (path a in *Figure 1*).[8–11] This association was stronger for lifetime BMI than adult BMI, which is, as would be expected, given that genotypes should have life-long influence on risk factor levels.[35] Our analysis using the *FTO* genotype as an instrument for life-long BMI levels is therefore likely to be less biased by attenuation by errors than those based on a single measurement of BMI. In addition, *FTO* polymorphism was associated with parental BMI, as would be expected, given that offspring share all genes with their parents (50% with each parent). The slightly stronger association with maternal than paternal BMI may reflect intrauterine influences of maternal obesity programming increased life-long obesity risk in the offspring, non-paternity generating attenuated paternal associations, or random variation in associations.[36–38]

Secondly, *FTO* polymorphism was not associated with factors such as sex, age, early or adult socioeconomic circumstances, educational attainment, smoking, or alcohol consumption. Thus, these common confounding factors are unlikely to distort the instrumental variables analysis of the effect of BMI on atherosclerosis risk (path b in *Figure 1*), as has been demonstrated previously.[39] Thirdly, as the *FTO* genotype determines the increased risk of higher BMI levels from early life onwards, reverse causality where subclinical atheroma affects risk factor levels was largely eliminated.

A full understanding of the function of the *FTO* gene is undergoing further exploration. However, both high BMI and type 2 diabetes contribute to atherosclerotic vascular changes,[40–43] and an additive effect of the *FTO* variant rs9939609 on BMI has been replicated in at least 13 unselected independent population studies with 38 759 participants.[8] In the Avon Longitudinal Study of Parents and Children (ALSPAC) offspring, the variant was shown to have a specific association with fat mass.[8] In genome wide studies, the *FTO* gene has been identified to predispose to type 2 diabetes via an effect on BMI.[8] Furthermore, it has been shown that the *FTO* gene is associated with insulin resistance, hyperglycaemia, dyslipidaemia, and hypertension, as one would predict based on observational evidence of the association of BMI with these outcomes,[44] and of randomized trials of the effect of weight change interventions on these outcomes.[44] These findings suggest that greater adiposity is indeed causally related to these outcomes and that the observational studies for these associations provide

valid estimates of effect. Furthermore, these findings provide some support that *FTO* polymorphism is a valid instrument for adiposity. If it were not a valid instrument of adiposity, then one would not expect such accurate prediction of the causal effect of adiposity on outcomes that are generally widely accepted as being causally related to greater adiposity,[45] and that alter in relation to changes in BMI in randomized controlled trials of drug and surgery treatments that reduce BMI.[46–48]

Study limitations

Limitations of Mendelian randomization testing have been discussed in detail previously.[5] It is possible that *FTO* has other direct phenotypic effects in addition to those on BMI, and it is these that influence the outcomes. Furthermore, the expression of *FTO* during early-life development could trigger a canalizing response that would mitigate the effects of increased adiposity. Studies utilizing additional independent genetic variants related to BMI, and investigations of when, during development, the variants begin to influence adiposity, could help resolve these issues. It could be argued that, as adjustment for BMI did not abolish the *FTO*-outcome associations, this suggests that there are indeed alternative pathways linking *FTO* to the outcomes. However, it must be remembered that even the repeat measures of BMI utilized in our lifetime BMI score will not be an accurate indicator of true lifetime adiposity, and a residual effect of *FTO* after adjustment for our lifetime BMI measure would be expected even if the entire effect of *FTO* was mediated through adiposity.

In this study, the wider confidence intervals for effect estimates from instrumental variables analysis compared with those obtained from the standard regression models demonstrate a drawback. Body mass index is a complex trait, and the small proportion of variation in BMI explained by the genetic instrument based on a single gene leads to the much lower precision of the instrumental variables analysis estimates compared with the estimates from standard regression models. Thus, the instrumental variable estimates are typically less efficient than the standard regression estimates if there is no confounding, reverse causality, or measurement error, but unlike the standard regression estimators the instrumental variables estimates should remain consistent when these are present. Besides supporting the role of BMI as a contributing factor to CIMT and various atherosclerotic risk factors, our findings suggest that the true influence of BMI on these factors might be underestimated in cohort studies. However, larger samples and/or stronger genetic instruments are needed before this conclusion can be convincingly supported by statistically robust results after correction for multiple testing. Furthermore, as the function of *FTO* is not fully known, we cannot be certain that the *FTO* polymorphism conferred effect on atherosclerosis risk factors only through adiposity.

Conclusion

Mendelian randomization approach has different strengths and limitations than conventional analysis. We found evidence that genetic variants in the *FTO* gene were not related to potential confounding factors; that they were strongly associated with lifetime BMI levels; and that the findings on the effects of lifetime BMI on CIMT and atherosclerosis risk factors as obtained from Mendelian randomization and standard regression models were converging. These results suggest that Mendelian randomization approach with *FTO* genotype data indeed have the potential of complementing existing evidence on the effects of lifetime BMI on atherosclerosis risk in large cohorts of young adults.

Supplementary material

Supplementary material is available at *European Heart Journal* online.

Conflict of interest: none declared.

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References

1. Davey Smith G, Ebrahim S. Data dredging, bias, or confounding. *Br Med J* 2002; **325**:1437–1438.
2. Duff GL, McMillan GC. Pathology of atherosclerosis. *Am J Med* 1951;**11**:92–108.
3. Davey Smith G, Ebrahim S. 'Mendelian randomization': can genetic epidemiology contribute to understanding environmental determinants of disease? *Int J Epidemiol* 2003;**32**:1–22.
4. Ebrahim S, Davey Smith G. Mendelian randomization: can genetic epidemiology help redress the failures of observational epidemiology? *Hum Genet* 2007;**123**: 15–33.
5. Lawlor DA, Harbord RM, Sterne JA, Timpson N, Davey Smith G. Mendelian randomization: using genes as instruments for making causal inferences in epidemiology. *Stat Med* 2007;**27**:1133–1163.
6. Hingorani A, Humphries S. Nature's randomised trials. *Lancet* 2005;**366**: 1906–1908.
7. Raitakari OT, Juonala M, Kähönen M, Taittonen L, Laitinen T, Mäki-Torkko N, Järvisalo MJ, Uhari M, Jokinen E, Rönnemaa T, Åkerblom HK, Viikari JS. Cardiovascular risk factors in childhood and carotid artery intima-media thickness in adulthood: the Cardiovascular Risk in Young Finns study. *JAMA* 2003;**290**:2277–2283.
8. Frayling TM, Timpson NJ, Weedon MN, Zeggini E, Freathy RM, Lindgren CM, Perry JR, Elliott KS, Lango H, Rayner NW, Shields B, Harries LW, Barrett JC, Ellard S, Groves CJ, Knight B, Patch AM, Ness AR, Ebrahim S, Lawlor DA, Ring SM, Ben-Shlomo Y, Järvelin MR, Sovio U, Bennett AJ, Melzer D, Ferrucci L, Loos RJ, Barroso I, Wareham NJ, Karpe F, Owen KR, Cardon LR, Walker M, Hitman GA, Palmer CN, Doney AS, Morris AD, Davey Smith G, Hattersley AT, McCarthy MI. A common variant in the FTO gene is associated with body mass index and predisposes to childhood and adult obesity. *Science* 2007;**316**:889–894.
9. Scuteri A, Sanna S, Chen WM, Uda M, Albai G, Strait J, Najjar S, Nagaraja R, Orrú M, Usala G, Dei M, Lai S, Maschio A, Busonero F, Mulas A, Ehret GB, Fink AA, Weder AB, Cooper RS, Galan P, Chakravarti A, Schlessinger D, Cao A, Lakatta E, Abecasis GR. Genome-wide association scan shows genetic variants in the FTO gene are associated with obesity-related traits. *PLoS Genet* 2007;**3**:e115.
10. Dina C, Meyre D, Gallina S, Durand E, Körner A, Jacobson P, Carlsson LM, Kiess W, Vatin V, Lecoeur C, Delplanque J, Vaillant E, Pattou F, Ruiz J, Weill J, Levy-Marchal C, Horber F, Potoczna N, Hercberg S, Le Stunff C, Bougnères P, Kovacs P, Marre M, Balkau B, Cauchi S, Chèvre JC, Froguel P. Variation in FTO contributes to childhood obesity and severe adult obesity. *Nat Genet* 2007;**39**: 724–726.

11. Scott LJ, Mohlke KL, Bonnycastle LL, Willer CJ, Li Y, Duren WL, Erdos MR, Stringham HM, Chines PS, Jackson AU, Prokunina-Olsson L, Ding CJ, Swift AJ, Narisu N, Hu T, Pruim R, Xiao R, Li XY, Conneely KN, Riebow NL, Sprau AG, Tong M, White PP, Hetrick KN, Barnhart MW, Bark CW, Goldstein JL, Watkins L, Xiang F, Saramies J, Buchanan TA, Watanabe RM, Valle TT, Kinnunen L, Abecasis GR, Pugh EW, Doheny KF, Bergman RN, Tuomilehto J, Collins FS, Boehnke M. A genome-wide association study of type 2 diabetes in Finns detects multiple susceptibility variants. *Science* 2007;**316**:1341–1345.
12. Cardon LR, Palmer LJ. Population stratification and spurious allelic association. *Lancet* 2003;**361**:598–604.
13. Åkerblom HK, Uhari M, Pesonen E, Dahl M, Kaprio EA, Nuutinen EM, Uhari M, Pietikäinen M, Salo MK, Aromaa A, Kannas L, Keltikangas-Järvinen L, Kuusela V, Räsänen L, Rönnemaa T, Knip M, Telama R, Välimäki I, Pyörälä K, Viikari J. Cardiovascular Risk in Young Finns. *Ann Med* 1991;**23**:35–39.
14. Livak KJ. Allelic discrimination using fluorogenic probes and the 5′ nuclease assay. *Genet Anal* 1999;**14**:143–149.
15. Yang X, Telama R, Leskinen E, Mansikkaniemi K, Viikari J, Raitakari OT. Testing a model of physical activity and obesity tracking from youth to adulthood: the cardiovascular risk in young Finns study. *Int J Obes (Lond)* 2007;**31**:521–527.
16. Kivimäki M, Lawlor DA, Davey Smith G, Keltikangas-Järvinen L, Elovainio M, Vahtera J, Pulkki-Råback L, Taittonen L, Viikari JS, Raitakari OT. Early socioeconomic position and blood pressure in childhood and adulthood: the Cardiovascular Risk in Young Finns study. *Hypertension* 2006;**47**:39–44.
17. Uhari M. Evaluation of the measurement of children's blood pressure in an epidemiological multicentre study. *Acta Paediatr Scand Suppl* 1985;**318**:79–88.
18. Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. *Clin Chem* 1972;**18**:499–502.
19. Juonala M, Viikari JS, Laitinen T, Marniemi J, Helenius H, Rönnemaa T, Raitakari OT. Interrelations between brachial endothelial function and carotid intima-media thickness in young adults: the cardiovascular risk in young Finns study. *Circulation* 2004;**110**:2918–2923.
20. Kivimäki M, Davey Smith G, Juonala M, Ferrie JE, Keltikangas-Järvinen L, Elovainio M, Pulkki-Råback L, Vahtera J, Leino M, Viikari JS, Raitakari OT. Socioeconomic position in childhood and adult cardiovascular risk factors, vascular structure and function: the Cardiovascular Risk in Young Finns study. *Heart* 2006;**92**:474–480.
21. Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC. Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. *Diabetologia* 1985;**28**:412–419.
22. StatisticsFinland. *Classification of Occupations*. Helsinki: Statistics Finland; 1987.
23. Timpson NJ, Lawlor DA, Harbord RM, Gaunt TR, Day IN, Palmer LJ, Hattersley AT, Ebrahim S, Lowe GD, Rumley A, Davey Smith G. C-reactive protein and its role in metabolic syndrome: Mendelian randomisation study. *Lancet* 2005;**366**:1954–1959.
24. Davey Smith G, Lawlor DA, Harbord R, Timpson N, Rumley A, Lowe GD, Day IN, Ebrahim S. Association of C-reactive protein with blood pressure and hypertension. Life course confounding and Mendelian randomization tests of causality. *Arterioscler Thromb Vasc Biol* 2005;**25**:1051–1056.
25. Lawlor DA, Timpson N, Ebrahim S, Day IN, Davey Smith G. The association of oestrogen receptor alpha-haplotypes with cardiovascular risk factors in the British Women's Heart and Health Study. *Eur Heart J* 2006;**27**:1597–1604.
26. Kivimäki M, Lawlor DA, Eklund C, Davey Smith G, Hurme M, Lehtimäki T, Viikari JSA, Raitakari OT. Mendelian randomization suggests no causal association between C-reactive protein and carotid intima-media thickness in the young Finns study. *Arterioscler Thromb Vasc Biol* 2007;**27**:978–979.
27. Sacerdote C, Guarrera S, Davey Smith G, Grioni S, Krogh V, Masala G, Mattiello A, Palli D, Panico S, Tumino R, Veglia F, Matullo G, Vineis P. Lactase persistence and bitter taste response: Instrumental variables and Mendelian randomization in epidemiologic studies of dietary factors and cancer risk. *Am J Epidemiol* 2007;**166**:576–581.
28. Keavney B, Danesh J, Parish S, Palmer A, Clark S, Youngman L, Delépine M, Lathrop M, Peto R, Collins R. Fibrinogen and coronary heart disease: test of causality by 'Mendelian randomization'. *Int J Epidemiol* 2006;**35**:935–943.
29. Casas JP, Shah T, Cooper J, Hawe E, McMahon AD, Gaffney D, Packard CJ, O'Reilly DS, Juhan-Vague I, Yudkin JS, Tremoli E, Margaglione M, Di Minno G, Hamsten A, Kooistra T, Stephens JW, Hurel SJ, Livingstone S, Colhoun HM, Miller GJ, Bautista LE, Meade T, Sattar N, Humphries SE, Hingorani AD. Insight into the nature of the CRP-coronary event association using Mendelian randomization. *Int J Epidemiol* 2006;**35**:922–931.
30. Casas JP, Bautista LE, Smeeth L, Sharma P, Hingorani AD. Homocysteine and stroke: evidence on a causal link from Mendelian randomisation. *Lancet* 2005;**365**:224–232.
31. Davey Smith G, Harbord R, Milton J, Ebrahim S, Sterne JA. Does elevated plasma fibrinogen increase the risk of coronary heart disease? Evidence from a meta-analysis of genetic association studies. *Arterioscler Thromb Vasc Biol* 2005;**25**:2228–2233.
32. Greenland S. An introduction to instrumental variables for epidemiologists. *Int J Epidemiol* 2000;**29**:722–729.
33. Stock JH, Wright JH, Yogo M. A survey of weak instruments and weak identification in generalized method of moments. *J Bus Econ Stat* 2002;**20**:518–529.
34. Staiger D, Stock JH. Instrumental variables regression with weak instruments. *Econometrica J Econ Soc* 1997;**65**:557–586.
35. Kivimäki M, Lawlor DA, Davey Smith G, Eklund C, Hurme M, Lehtimaki T, Viikari JSA, Raitakari OT. Variants in the CRP gene as a measure of lifelong differences in average C-reactive protein levels: the Cardiovascular Risk in Young Finns study, 1980-2001. *Am J Epidemiol* 2007;**166**:760–764.
36. Hillier TA, Pedula KL, Schmidt MM, Mullen JA, Charles MA, Pettitt DJ. Childhood obesity and metabolic imprinting: the ongoing effects of maternal hyperglycemia. *Diabetes Care* 2007;**30**:2287–2292.
37. Dabelea D, Hanson RL, Lindsay RS, Pettitt DJ, Imperatore G, Imperatore G, Gabir MM, Roumain J, Bennett PH, Knowler WC. Intrauterine exposure to diabetes conveys risks for type 2 diabetes and obesity: a study of discordant sibships. *Diabetes* 2000;**49**:2208–2211.
38. Kivimäki M, Lawlor DA, Davey Smith G, Elovainio M, Jokela M, Keltikangas-Järvinen L, Viikari JS, Raitakari OT. Substantial intergenerational increases in body mass index are not explained by the fetal overnutrition hypothesis: the Cardiovascular Risk in Young Finns study. *Am J Clin Nutr* 2007;**86**:1509–1514.
39. Davey Smith G, Lawlor DA, Harbord R, Timpson N, Day I, Ebrahim S. Clustered environments and randomized genes: a fundamental distinction between conventional and genetic epidemiology. *PLoS Med* 2007;**4**:e352.
40. Lee WL, Cheung AM, Cape D, Zinman B. Impact of diabetes on coronary artery disease in women and men: a meta-analysis of prospective studies. *Diabetes Care* 2000;**23**:962–968.
41. Van Gaal LF, Mertens IL, De Block CE. Mechanisms linking obesity with cardiovascular disease. *Nature* 2006;**444**:875–880.
42. Katagiri H, Yamada T, Oka Y. Adiposity and cardiovascular disorders: disturbance of the regulatory system consisting of humoral and neuronal signals. *Circ Res* 2007;**101**:27–39.
43. Reusch JE, Draznin BB. Atherosclerosis in diabetes and insulin resistance. *Diabetes Obes Metab* 2007;**9**:455–463.
44. Freathy RM, Timpson NJ, Lawlor DA, Pouta A, Ben-Shlomo Y, Ruokonen A, Ebrahim S, Shields B, Zeggini E, Weedon MN, Lindgren CM, Lango H, Melzer D, Ferrucci L, Paolisso G, Neville MJ, Karpe F, Palmer CN, Morris AD, Elliott P, Jarvelin MR, Davey Smith G, McCarthy MI, Hattersley AT, Frayling TM. Common variation in the FTO gene alters diabetes-related metabolic traits to extent expected, given its effect on BMI. *Diabetes* 2008;**57**:1419–1426.
45. Fourth Joint Task Force of the European Society of Cardiology and Other Societies on Cardiovascular Disease Prevention in Clinical Practice (Constituted by representatives of nine societies and by invited experts), Graham I, Atar D, Borch-Johnsen K, Boysen G, Burell G, Cifkova R, Dallongeville J, De Backer G, Ebrahim S, Gjelsvik B, Herrmann-Lingen C, Hoes A, Humphries S, Knapton M, Perk J, Priori SG, Pyorala K, Reiner Z, Ruilope L, Sans-Menendez S, Scholte op Reimer W, Weissberg P, Wood D, Yarnell J, Zamorano JL; Other experts who contributed to parts of the guidelines; Walma E, Fitzgerald T, Cooney MT, Dudina A;, European Society of Cardiology (ESC) Committee for Practice Guidelines (CPG), Vahanian A, Camm J, De Caterina R, Dean V, Dickstein K, Funck-Brentano C, Filippatos G, Hellemans I, Kristensen SD, McGregor K, Sechtem U, Silber S, Tendera M, Widimsky P, Zamorano JL, Hellemans I, Altiner A, Bonora E, Durrington PN, Fagard R, Giampaoli S, Hemingway H, Hakansson J, Kjeldsen SE, Larsen ML, Mancia G, Manolis AJ, Orth-Gomer K, Pedersen T, Rayner M, Sammut M, Schneiderman N, Stalenhoef AF, Tokgözoglu L, Wiklund O, Zampelas A European guidelines on cardiovascular disease prevention in clinical practice: executive summary. *Eur Heart J* 2007;**28**:2375–2414. Other experts who contributed to parts of the guidelines. European Society of Cardiology (ESC) Committee for Practice Guidelines (CPG).
46. Van Gaal LF, Rissanen AM, Scheen AJ, Ziegler O, Rossner S. Effects of the cannabinoid-1 receptor blocker rimonabant on weight reduction and cardiovascular risk factors in overweight patients: 1-year experience from the RIO-Europe study. *Lancet* 2005;**365**:1389–1397.
47. Kim SH, Lee YM, Jee SH, Nam CM. Effect of sibutramine on weight loss and blood pressure: a meta-analysis of controlled trials. *Obes Res* 2003;**11**:1116–1123.
48. Colquitt J, Clegg A, Loveman E, Royle P, Sidhu MK. Surgery for morbid obesity. *Cochrane Database Syst Rev* 2005; CD003641.

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