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Using genetic loci to understand the relationship between adiposity and psychological distress: a Mendelian Randomization study in the Copenhagen General Population Study of 53 221 adults

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Abstract. Lawlor DA, Harbord RM, Tybjaerg-Hansen A, Palmer TM, Zacho J, Benn M, Timpson NJ, Smith GD, Nordestgaard BG (MRC Centre for Causal Analyses in Translational Epidemiology, University of Bristol, UK; The Department of Social Medicine, University of Bristol, UK; Department of Clinical Biochemistry, Rigshospitalet, Copenhagen, Denmark; The Copenhagen General Population Study, Herlev Hospital, Copenhagen, Denmark; Copenhagen University Hospital, Faculty of Health Sciences, University of Copenhagen, Denmark; Department of Clinical Biochemistry, Herlev Hospital, Copenhagen, Denmark) Using genetic loci to understand the relationship between adiposity and psychological distress: a Mendelian Randomization study in the Copenhagen General Population Study of 53 221 adults. *J Intern Med* 2011; **269**: 525–537.

Objective. We used genetic variants that are robustly associated with adiposity to examine the causal association of adiposity with psychological distress.

Methods. We examined the association of adiposity with psychological distress in a large (N = 53 221) general population cohort of 20- to 99-year-old adults from Copenhagen, Denmark. Psychological distress was assessed using four questions that asked about: feeling stressed; not accomplishing very much; wanting to give up; and regular use of antidepressants/sedatives. We used the genetic loci *FTO* rs9939609 and *MC4R* rs17782313 as instrumental variables for adiposity quantified by body mass index (BMI) and waist to hip ratio (WHR).

Results. In conventional multivariable analyses, BMI and WHR were positively associated with distress. For example, the odds ratio of reporting not accomplishing for each additional standard deviation increase for BMI was 1.11 (95% CI: 1.09, 1.13) and for WHR was 1.10 (95% CI: 1.08, 1.13) in the fully adjusted analyses. In contrast, instrumental variable analyses showed an inverse association of adiposity on distress; corresponding odds ratio in instrumental variable analyses was 0.64 (95% CI: 0.46, 0.89) for BMI and 0.49 (95% CI: 0.25, 0.94) for WHR (P-values for difference between the two approaches both = 0.001).

Conclusion. The inverse associations of adiposity and psychological distress when genetic variants are used as instrumental variables could be explained by biological pathways linking adiposity and distress. The positive associations of adiposity with distress in multivariable analyses might be explained by residual confounding or reverse causality.

Keywords: BMI, genetic variation, mendelian randomization, psychological distress, waist to hip ratio.

Introduction

An association between greater levels of adiposity and psychological distress or disorders, including depression and anxiety, has been reported in several publications, but the direction and nature of these associations remains unclear [1, 2]. Some studies have reported that individuals with greater adiposity or obesity are at increased risk of psychological distress and disorders [3–6]. Others have reported no

association [7, 8], and some have reported negative associations (i.e. greater adiposity associated with reduced risk of distress and indeed in some studies of suicide) [9–13]. Furthermore, a number of studies have considered psychological distress to be a risk factor for obesity (rather than the other way round) and again in these results have been conflicting [14–19]. A recent systematic review of cohort studies found a positive association of obesity with depression and also of depression with obesity [20]. However, this review did not include a very recent study with repeat measurements of adiposity and depression [19], and of the 15 studies identified and included only four scored highly on the quality assessment undertaken by the authors. Observational studies may produce incorrect causal estimates because of the potential for residual confounding, reverse causality and reporting bias.

Several mechanisms could explain the potential bidirectional and conflicting associations implied by the findings from previous research. Both social and biological mechanisms are likely to be involved, and these may result in associations in opposite directions. First, psychological distress and disorders are associated with eating disorders, both over- and under-consumption, which could result in increases or decreases in weight in those with these conditions. Second, in societies where obesity is stigmatized, this stigmatization may lead to increased risk of psychological distress in those who are obese [21]. Third, commonly used treatments for depression have known side-effects that result in weight gain (tricyclic antidepressants], weight loss (selective serotonin reuptake inhibitors, SSRI) or both (short- and long-term effects of SSRI). Fourth, greater adiposity is associated with increased insulin resistance and circulating free fatty acids and tryptophan, which in turn may increase brain serotonin and/or influence the hypothalamic–pituitary stress axes and consequently decrease symptoms of psychological distress in those who are more obese [22–25]. Finally, residual confounding, by socioeconomic position, smoking, alcohol, physical activity and other lifestyle characteristics that have either been imprecisely measured or gone unmeasured, could explain the associations seen in some previous studies [26, 27]. Reverse causality or reporting bias (for example, with those who are more obese feeling they ought to report being sadder) could also explain these previous associations.

One way to shed light on the currently conflicting evidence is to complete a Mendelian randomization study, in which genetic variants that have been reliably associated with adiposity are used as instrumental variables for adiposity in estimating the causal effect of it on psychological distress [28, 29]. In this approach, the variability in adiposity that is influenced by genotype is related to outcomes. Because genetic variants are unlikely to be associated with characteristics that commonly confound observational epidemiology, any association of adiposity-related genotype with psychological distress is unlikely to be explained by residual confounding [28–30]. Furthermore, germline genotype is allocated at conception, and therefore, reverse causality (psychological distress influencing adiposity genotype) is not possible. Thus, this approach provides a causal estimate of the association of adiposity with psychological outcomes that is less likely to be affected by confounding factors and biased by reverse causality than the causal estimate from a multivariable analysis [28–30].

The aim of this study was to use two genetic variants that have been reliably associated with variation in body mass index (BMI), waist circumference/waist:hip ratio (WHR) and fat mass [31–33] as instrumental variables to examine the relationship of adiposity with psychological distress in a very large cohort (larger than any previous studies) and compare the estimates from this instrumental variable (Mendelian randomization) approach to those from conventional multivariable regression.

Methods

We used data from the Copenhagen General Population Study, a cross-sectional study that aims to eventually recruit 100 000 participants representative of the general population and collect genotypic and phenotypic data of relevance to a wide range of health-related problems. Individuals are randomly selected from the national Danish Civil Registration System and have to be aged above 20 years and resident in greater Copenhagen. Recruitment began in 2003 and is still ongoing. At the time of genotyping for the present study, 55 666 individuals had been included in the study, of whom 53 221 (96%) had complete data on all variables included in any analyses presented here. Table S1 shows the extent of missing data for each variable; missing data varied from 0 to 1.5% for any single variable. Our analyses are based on the 53 221 participants with complete data on all variables. Additional study details have been previously published [34–36].

Outcome assessment

Psychological distress was assessed by three questions that aimed to elicit symptoms of depression or anxiety:

Do you often feel nervous or stressed?

Do you have the feeling that you have not accomplished very much recently?

Do you feel like giving up life?

Each response was coded 1 for response yes and 0 for response no. In addition, participants were asked whether they currently used (daily or most days) '*Antidepressants, sedatives or relaxing pills*' (referred to as 'antidepressant medication' throughout the remainder of the paper). This was also coded 1 for response yes and 0 for response no.

Measures of adiposity

Measurements were completed by trained staff at one clinic centre. Weight was measured without shoes and in light clothing to the nearest 0.1 kg on Soehnle Professional scales. Height was measured to the nearest 0.1 cm with a Seca stadiometer. Waist (to nearest mm) was measured at the midpoint between the lower end of the rib cage and the upper part of the pelvic bone and hip (to nearest mm) at the largest circumference below the waist.

Genotyping

Genotyping was conducted blind to any phenotypic data. The ABI PRISM 7900HT Sequence Detection System (Applied Biosystems Inc., Foster City, CA, USA) was used to genotype the *FTO* rs9939609 and *MC4R* rs17782313 loci using TaqMan assays. Genotyping was verified by DNA sequence in at least 30 individuals with each genotype. We performed reruns twice, and 99.96% of all available participants were genotyped. Deviation from Hardy Weinberg equilibrium was investigated using a chi-squared test.

Covariables

Participants completed a questionnaire that recorded smoking (coded as ever versus never) and amounts smoked and ages of starting and quitting, which have been used to calculate pack-years of cigarettes smoked for each participant. Participants were also asked about alcohol consumption (coded as heavy drinking >36 g per week or not), leisure time physical activity (coded as 0–2 h moderate activity; 2–4 h moderate activity; >4 h moderate activity or 2–4 h vigorous activity; >4 h vigorous activity per week), annual income (coded as <100 000 Kr; 100 000–399 000 Kr; 400 000–599 000 Kr; ≥600 000 Kr (100 000 Kr – 17 000 US$/£10 500) and education (0–9; 10–12; ≥13 year of completed education) in this questionnaire.

Statistical analyses

We tabulated means and standard deviations of continuous variables and numbers and percentage of categorical variables by each of the outcomes and also by genotype, with *t*/*F*-tests and chi-squared tests used to test for differences between groups.

Because of the wide age range (20–99 years) included in the study, we generated z-scores (standard deviation scores) for BMI and WHR internally standardized for age (in 5-year age bands) and sex. We undertook two approaches to examining the associations of BMI and WHR with each outcome.

Multivariable analyses

The first set of analyses used multivariable logistic regression. In these analyses, we controlled for potential confounding by age, gender, smoking, alcohol consumption, physical activity, income and education. We then additionally adjusted for our proposed instrumental variables (the two genetic variants), under the assumption that if the multivariable analyses were confounded or biased, this effect would be expected to be exacerbated by adjustment for valid instrumental variables.

We hypothesized that if greater adiposity was related to increased risk of psychological distress because of stigmatization of obesity in society, positive associations might be stronger in women rather than men, at younger ages rather than older ages and that the association might show a threshold effect rather than increasing across the whole distribution of BMI or WHR. We examined whether associations were similar in males and females by stratifying on gender and comparing multivariable associations between males and females, and by testing for evidence of statistical interaction by gender using a likelihood ratio test. We examined interactions with age by separating age into five age categories (20–39.99; 40–49.99; 50–59.99; 60–69.99; ≥70), comparing multivariable

associations between each of these age categories, and testing for evidence of statistical interaction using a likelihood ratio test. We examined whether the associations were linear across the distribution of adiposity markers by two methods. First, we added a quadratic term to the regression models for both BMI and WHR and compared this to the linear model (without the quadratic term) using a likelihood ratio test. Second, we divided BMI into WHO categories of underweight (BMI <18.5 kg/m^2), healthy weight (18.5–24.9 kg/m^2), overweight (25–30 kg/m^2) and obesity (>30 kg/m^2) and used these categories as the exposure variable.

Instrumental variable analyses

In the second set of analyses, we used the *FTO* rs9939609 and *MC4R* rs17782313 loci as instrumental variables for the causal (unconfounded and without reverse causality) effect of mean differences in BMI and WHR on outcomes (i.e. modelling BMI and WHR as continuous variables in these analyses). We used the control function estimator for our main instrumental variable analyses [37, 38] and checked this approach by also completing analyses using a maximum quasi-likelihood estimator (qvf command in Stata) [37] and logistic structural mean models (LSMM) [39]. The control function estimator has been shown to be equivalent to the structural mean model estimator when the risk factor of interest (here BMI and WHR) is approximately normally distributed [40], as in our study. We present instrumental variable analyses using each of the two SNPs independently, using the genotypes as categories (two indicator variables for each genotype), and then using their categories jointly [41]. We used a Sargan type test of over-identification. Testing the 'over-identification restriction' checks the joint validity of multiple instruments by testing whether they give the same estimates when used singly or in linear combination. Finally, we repeated the instrumental variable analyses with BMI in categories of underweight, normal weight, overweight and obese. In these analyses, we also used the control function method, with BMI categories regressed on genotype in the first (ologit) regression.

Comparing multivariable and instrumental variable estimates

To compare the results of the multivariable (fully adjusted) association with that from the instrumental variable analysis, we used 1000 bootstrap replications [42] to estimate standard errors of the instrumental variable estimates of the log odds ratios and also to estimate standard errors of their differences from the multivariable estimates that account for the correlation between the two sets of estimates. We calculated confidence intervals and *P*-values from these standard errors based on a normal approximation for the sampling distribution of the log odds ratios, after checking the bootstrap distributions were close to normal using normal probability plots. All analyses were conducted in Stata/MP version 11.0 (StataCorp, TX, USA).

Results

Table S1 shows the characteristics of the cohort and Fig. S1 the prevalence and extent of overlap of the outcomes. Six per cent of participants reported taking antidepressants regularly, 7% that they felt like giving up living, 22% that they had accomplished nothing recently and 26% that they were nervous or stressed. One per cent of participants reported all four outcomes and 61% reported none. Mean BMI was 26.2 kg/m^2 and mean WHR was 0.874. Age and gender standardized BMI and WHR were only modestly associated with each other (Pearson's correlation coefficient = 0.48). The minor allele frequencies for *FTO* rs9939609 and *MC4R* rs17782313 were 0.40 and 0.25, respectively, and there was no evidence of departure from Hardy Weinberg equilibrium (Table S1).

Standard multivariable associations

Table S2 shows the association of BMI, WHR and covariables with each outcome. Women, those who had ever smoked and those who did little leisure time activity, were more likely to have any of the outcomes. All outcomes were less common in those who reported drinking more than 36 g of alcohol per week. With few exceptions, outcomes were more common amongst those with low income and low education. Those who reported feeling nervous or stressed were more likely to be from higher education groups, but this outcome was not associated with income. For all of the outcomes except feeling nervous or stressed, mean BMI and WHR were higher in those with, than without, the outcome. There was no strong evidence of an association between BMI and feeling nervous or stressed.

Table 1 shows the multivariable associations of BMI and WHR with outcomes. With the exception of the association of BMI with reporting feeling nervous or stressed, both anthropometric measurements were positively associated with all outcomes. With adjustment for potential confounding factors, there was

Table 1 *Multivariable association of body mass index and waist to hip ratio (as continuous variables) with psychological distress*

	Odds ratio (95% confidence interval) for each of outcome			
	Stressed/nervous	Not accomplishing	Feels like giving up	Antidepressant medication
Exposure = per age and sex standardized *z*-score body mass index				
Model 1	1.01 (0.99, 1.03)	1.22 (1.20, 1.24)	1.21 (1.17, 1.24)	1.14 (1.10, 1.18)
Model 2	0.97 (0.95, 0.99)	1.11 (1.09, 1.13)	1.10 (1.06, 1.13)	1.04 (1.01, 1.08)
Model 3	0.97 (0.95, 0.99)	1.13 (1.10, 1.15)	1.11 (1.06, 1.14)	1.06 (1.02, 1.09)
Exposure = per age and sex standardized *z*-score waist:hip ratio				
Model 1	1.08 (1.06, 1.11)	1.22 (1.19, 1.24)	1.22 (1.18, 1.26)	1.27 (1.23, 1.31)
Model 2	1.05 (1.03, 1.07)	1.10 (1.08, 1.13)	1.11 (1.08, 1.15)	1.16 (1.12, 1.20)
Model 3	1.06 (1.03, 1.08)	1.10 (1.08, 1.14)	1.12 (1.08, 1.16)	1.18 (1.13, 1.22)

N = 53 221 with complete data on any variable included in any analyses.
Model 1: Control for age and gender by use of age and gender standardized z-scores for each exposure.
Model 2: As model 1 plus additional adjustment for smoking, alcohol consumption, physical activity, income and education.
Model 3: As model 2 plus additional adjustment for *FTO* (rs9939609) and *MC4R* (rs17782313).
In sensitivity analyses where we adjusted for pack-years of smoking, in addition to other potential confounders, amongst the 44 100 with these data, results were essentially the same as those presented in model 2 of this table.

substantial amounts of attenuation of all of these associations (most reduced by approximately 50%), but all remained consistent with a positive association. Most of this attenuation was the result of adjustment for education, income and smoking, with alcohol and physical activity having relatively little attenuating effect. With additional adjustment for the genetic variants (our instrumental variables), positive associations strengthened slightly for some associations, but on the whole, this additional adjustment did not markedly alter the confounder-adjusted associations. Additional adjustment of BMI for WHR and *vice versa* did not substantively alter any of the associations presented for model 2 in Table 1 (results available from authors on request).

For both BMI and WHR, there was evidence of non-linear associations with most outcomes (all *P*-values nonlinear associations ≤0.02). However, these non-linear associations were not driven by a threshold effect of stronger associations at the upper obese end of the distributions, but rather by 'J' shaped association with greater odds of outcomes in those who were at the extreme lower (as well as upper) end of the distribution for both BMI and WHR. This is illustrated with BMI categories in Table 2. When we removed the small proportion (<1%) of participants who were classified as underweight by BMI from the analyses, associations of BMI and WHR with all outcomes (except BMI with stress for which there was no association) were linear (all quadratic terms *P*-values > 0.1).

Table S3 shows the multivariable associations stratified by gender. There was little evidence that any of the multivariable analyses differed between men and women (*P*-values for interaction > 0.1), except that BMI was more strongly associated with feeling that little was accomplished in women than in men (*P*-value for interaction = 0.005). Table S4 shows the multivariable associations stratified by age. There was no evidence that any of the multivariable analyses differed by age (*P*-values for interaction > 0.3), except for a weaker association of BMI with wanting to give up in those aged 60 or older (*P*-value for interaction = 0.04) and weaker associations of BMI and WHR with use of antidepressants in those aged 60 or older (*P*-values for interaction 0.01 and 0.005, respectively).

Genetic instrumental variable associations

Table 3 shows the association of each genotype with the four outcome measurements, BMI and WHR, and with potential confounding factors. There was no strong evidence of associations of any confounding factors with the genetic loci. *FTO* rs9939609 and *MC4R* rs17782313 were associated with BMI and WHR. For both loci, the pattern followed an additive per allele pattern of association with BMI. For *MC4R*, WHR was greatest in those who were heterozygotes compared to the two homozgote groups. In all instrumental variable analyses, we have used categories of genotype (indicator variables), which do not assume any specific pattern of association. With the

Table 2 *Multivariable association of body mass index categories with psychological distress*

	Odds ratio (95% confidence interval) for each of outcome			
	Stressed/nervous	Not accomplishing	Feels like giving up	Antidepressant medication
Model 1				
Underweight *N* = 407	1.46 (1.20, 1.81)	1.44 (1.14, 1.80)	1.80 (1.31, 2.47)	1.88 (1.34, 2.63)
Healthy Weight *N* = 22594	1	1	1	1
Overweight *N* = 21434	0.99 (0.95, 1.04)	1.12 (1.06, 1.17)	1.11 (1.02, 1.20)	1.16 (1.06, 1.27)
Obese *N* = 8786	1.07 (1.01, 1.13)	1.66 (1.57, 1.76)	1.73 (1.58, 1.90)	1.48 (1.34, 1.64)
p-linear trend[a]	0.31	<0.001	<0.001	<0.001
p-nonlinear[a]	0.0002	<0.001	<0.001	<0.001
Model 2				
Underweight *N* = 407	1.40 (1.14, 1.72)	1.26 (1.00, 1.59)	1.57 (1.14, 2.18)	1.68 (1.19, 2.36)
Healthy Weight *N* = 22594	1	1	1	1
Overweight *N* = 21434	0.95 (0.91, 0.99)	1.05 (1.00, 1.10)	1.03 (0.95, 1.11)	1.08 (1.00, 1.18)
Obese *N* = 8786	0.93 (0.88, 0.99)	1.31 (1.24, 1.40)	1.33 (1.21, 1.46)	1.18 (1.06, 1.32)
p-linear trend[a]	0.001	<0.001	<0.001	0.01
p-nonlinear[a]	0.02	<0.001	<0.001	0.007
Model 3				
Underweight N = 407	1.40 (1.14, 1.72)	1.25 (1.00, 1.59)	1.58 (1.14, 2.18)	1.68 (1.19, 2.36)
Healthy Weight *N* = 22594	1	1	1	1
Overweight *N* = 21434	0.95 (0.91, 1.00)	1.05 (1.00, 1.10)	1.03 (0.95, 1.12)	1.09 (1.00, 1.19)
Obese *N* = 8786	0.94 (0.88, 0.99)	1.32 (1.24, 1.41)	1.33 (1.21, 1.47)	1.19 (1.07, 1.32)
p-linear trend[a]	0.001	<0.001	<0.001	0.01
p-nonlinear[a]	0.02	<0.001	<0.001	0.007

N = 53 221 with complete data on any variable included in any analyses.
[a]*P*-values obtained from likelihood ratio tests comparing the models with each category as indicator variables and then with the categories as an ordinal score.
Model 1: Control for age and gender by use of age and gender standardized z-scores for each exposure.
Model 2: As model 1 plus additional adjustment for smoking, alcohol consumption, physical activity, income and education.
Model 3: As model 2 plus additional adjustment for *FTO* (rs9939609) and *MC4R* (rs17782313).

exception of the association of *MC4R* rs17782313 with feeling like wanting to give up, the proportion with each outcome was least in those who were homozygotes for the adiposity increasing allele than other groups, although for some of the gene-outcome associations, *P*-values were consistent with the null hypothesis, and the associations were not always monotonic across the three genotype groups. When we adjusted these genotype-outcome associations for BMI, WHR or both together the point estimates and *P*-values remained essentially the same as those presented in Table 3.

Table 4 shows the comparisons of the conventional multivariable associations with the instrumental variable estimates with BMI and WHR as continuous age and sex standardized variables. The first-stage *F*-statistic for instrumental variable analyses suggested that we generally had sufficiently strong instruments, except for the first-stage *F*-statistic for *MC4R* used alone (for BMI, values were 85, 24, 55 for *FTO*, *MC4R* and both, respectively; for WHR, values were 28, 5, 16 for *FTO*, *MC4R* and both, respectively). The over-identification test showed no strong evidence against the joint use of the genotypes as multiple instruments, because only the model for using anti-depressants under analysis 1 gave a small *P*-value of 0.04 (all other *P*-values ≥ 0.1). All of the instrumental variable analyses suggested an inverse association of both BMI and WHR with outcomes. We focus further comments regarding the instrumental variables analysis for BMI on those using both loci in combination (instrumental variables analysis 3 in Table 3), but note conclusions would be similar if we focused on analyses using *FTO* only (analyses 1) or *MC4R* only (analyses 2). For WHR, only analyses

Table 3 *Psychological distress outcomes, body mass index, waist to hip ratio and potential confounding characteristics by genotype* FTO *and* MC4R

	Mean (SD) or Number (%) by genotype			
	Major allele homozygotes	Heterozygotes	Minor allele homozygotes	*P*-value[a]
FTO rs9939609				
Number	18819	25729	8646	
Continuously measured characteristics (data are means (SD))				
Age	56.9 (13.4)	56.8 (13.4)	56.7 (13.4)	0.72
Body Mass Index[b]	−0.053 (0.959)	0.004 (1.006)	0.116 (1.053)	<0.0001
Waist to Hip ratio[b]	−0.024 (0.975)	−0.004 (0.996)	0.071 (1.044)	<0.0001
Categorical characteristics (data are numbers (%) with category)				
Stressed/nervous	4907 (26.1)	6743 (26.2)	2130 (24.6)	0.01
Not accomplishing	3966 (21.1)	5454 (21.2)	1735 (20.1)	0.07
Feels like giving up	1263 (6.7)	1711 (6.7)	540 (6.2)	0.32
Antidepressant medication	1017 (5.4)	1456 (5.7)	424 (4.9)	0.03
Female	10452 (56)	13990 (54)	4767 (55)	0.05
Ever smoked	11229 (60)	15518 (60)	5244 (61)	0.22
Alcohol > 36 g/week	13675 (73)	18537 (72)	6184 (72)	0.12
Physical inactivity < 4 h moderate activity/week	9769 (52)	13254 (52)	45130 (52)	0.48
Income > 600 000 Kr	3505 (19)	4813 (19)	1591 (18)	0.82
Education > 13 years	3209 (17)	4312 (17)	1463 (17)	0.72
MC4R rs17782313				
Number	30192	19784	3218	
Continuously measured characteristics (data are means (SD))				
Age	56.8 (13.4)	56.8 (13.5)	57.2 (13.3)	0.23
Body Mass Index[b]	−0.024 (0.988)	0.034 (1.013)	0.051 (1.011)	<0.0001
Waist to Hip ratio[b]	−0.010 (0.994)	0.019 (0.999)	−0.003 (1.005)	0.0049
Categorical characteristics (data are numbers (%) with category)				
Stressed/nervous	7976 (26.4)	4996 (25.2)	808 (25.1)	0.008
Not accomplishing	6417 (21.2)	4108 (20.8)	630 (19.6)	0.05
Feels like giving up	2019 (6.7)	1267 (6.4)	228 (7.1)	0.25
Antidepressant medication	1672 (5.5)	1058 (5.4)	167 (5.2)	0.53
Female	16540 (55)	10876 (55)	1798 (56)	0.49
Ever smoked	18147 (60)	11893 (60)	1951 (61)	0.84
Alcohol > 36 g/week	21817 (72)	14259 (72)	2320 (72)	0.90
Physical inactivity < 4 h moderate activity/week	15595 (52)	10342 (52)	1599 (50)	0.02
Income > 600 000 Kr	5653 (19)	3679 (19)	577 (18)	0.54
Education > 13 years	5078 (17)	3386 (17)	520 (16)	0.36

N = 53 221 with complete data on any variable included in any analyses.
[a] *P*-values calculated *P*-values calculated from *F*-test for continuous outcomes and chi-squared test for binary outcomes – each test the hypothesis of any difference between the groups (not assuming a linear trend).
[b] both body mass index and waist to hip ratio are age and sex standardized *z*-scores.

Table 4 *Comparison of conventional multivariable and instrumental variable (with genotype as instrument) associations of body mass index and waist to hip ratio (as continuous variables) with psychological distress*

	Multivariable association	IV analysis 1	*P*-value 1	IV analysis 2	*P*-value 2	IV analysis 3	*P*-value 3
Estimates of odds ratio of outcome per 1 age and sex standardized z-score increase in BMI							
Stress/nervous	0.97 (0.95, 0.99)	0.65 (0.46, 0.91)	0.032	0.36 (0.19, 0.69)	0.005	0.57 (0.42, 0.77)	0.001
Not accomplishing	1.11 (1.09, 1.13)	0.69 (0.47, 1.01)	0.016	0.48 (0.24, 0.96)	0.022	0.64 (0.46, 0.89)	0.001
Wanting to give up	1.10 (1.06, 1.13)	0.63 (0.34, 1.15)	0.067	0.67 (0.21, 2.09)	0.32	0.63 (0.37, 1.08)	0.054
Using antidepressants	1.04 (1.01, 1.08)	0.57 (0.29, 1.10)	0.093	0.49 (0.14, 1.70)	0.24	0.55 (0.31, 0.98)	0.033
Estimates of odds ratio of outcome per 1 age and sex standardized z-score increase in WHR							
Stress/nervous	1.05 (1.03, 1.07)	0.42 (0.23, 0.78)	0.015				
Not accomplishing	1.10 (1.08, 1.13)	0.49 (0.25, 0.94)	0.001				
Wanting to give up	1.11 (1.08, 1.15)	0.42 (0.13, 1.25)	0.069				
Using antidepressants	1.16 (1.12, 1.20)	0.30 (0.09, 0.99)	<0.001				

N = 53 221 with complete data on any variable included in any analyses.
BMI: body mass index; WHR: waist to hip ratio.
The multivariable analysis controls for age, gender, smoking, alcohol consumption, physical activity, income and education.
Instrumental variable analysis 1: Using *FTO* (rs9939609) genotypes as categories (two indicator variables) only; *P*-value 1 compares this to the multivariable analysis.
Instrumental variable analysis 2: Using *MC4R* (rs17782313) genotypes as categories (2 indicator variables) only; *P*-value 2 compares this to the multivariable analysis.
Instrumental variable analysis 3: Using both *FTO* (rs9939609) and *MC4R* (rs17782313) genotypes as categories (4 indicator variables); *P*-value 3 compares this to the multivariable analysis.
Note IV analyses with *MC4R* as an instrumental variable for waist to hip ratio (WHR) were not completed because of this being a weak instrument for WHR.
All p-values compare the multivariable association result with the instrumental variable results and were derived from bootstrapping.
These instrumental variables estimates were obtained using the control function estimator, but essentially identical results were obtained from a maximum quasi-likelihood estimator and logistic structural mean models.

using *FTO* alone are presented because of potential problems with weak instruments for *MC4R* with WHR and because the bootstrap distributions using *MC4R* as instrument for WHR were noticeably non-normal. The results demonstrate statistical evidence of a difference between the multivariable and instrumental variable analyses for both BMI and WHR with all outcomes except wanting to give up. For wanting to give up, the point estimates looked different between the instrumental variable and multivariable analyses (and consistent with all other outcomes), but confidence intervals were wide, and *P*-values were borderline compared with conventional 5% levels of statistical significance. When we adjusted the instrumental variable analyses of the association of BMI with outcomes for WHR, and *vice versa*, the results did not differ from those presented in Table 4.

Table 5 compares multivariable and instrumental variable analyses (using both genetic variants) for BMI categories. For all four outcomes, instrumental variable analyses showed those in the underweight category to have similar or elevated odds of outcome compared with the normal weight category, with no evidence of a difference compared with the multivariable analyses. For both overweight and obese categories, the odds of outcomes were reduced compared to the normal weight category for all four outcomes, with strong statistical evidence that these estimates differed from multivariable analyses.

Discussion

We found positive associations of BMI and WHR with psychological distress and increased odds of these outcomes in those who were overweight or obese, using conventional multivariable approaches. In contrast, we found inverse associations when the same relationships were examined using adiposity-related genotypes as instrumental variables. Consis-

Table 5 *Comparison of conventional multivariable and instrumental variable associations of body mass index categories with psychological distress*

	Multivariable-adjusted odds ratios (95% CI)	IV-adjusted odds ratios (95% CI)	*P*-value 1
Outcome = odds of stress/anxiety			
Underweight *N* = 407	1.40 (1.14, 1.72)	1.20 (0.86, 1.68)	0.76
Normal weight *N* = 22594	1	1	
Overweight *N* = 21434	0.95 (0.91, 0.99)	0.33 (0.15, 0.74)	0.006
Obese *N* = 8786	0.93 (0.88, 0.99)	0.27 (0.09, 0.79)	0.003
Outcome = odds of not accomplishing			
Underweight *N* = 407	1.26 (1.00, 1.59)	0.99 (0.69, 1.43)	0.85
Normal weight *N* = 22594	1	1	
Overweight *N* = 21434	1.05 (1.00, 1.10)	0.34 (0.14, 0.79)	<0.001
Obese *N* = 8786	1.31 (1.24, 1.40)	0.34 (0.11, 1.08)	<0.001
Outcome = odds of wanting to give up			
Underweight *N* = 407	1.57 (1.14, 2.18)	1.19 (0.67, 2.11)	0.62
Normal weight *N* = 22594	1	1	
Overweight *N* = 21434	1.03 (0.95, 1.11)	0.25 (0.06, 1.04)	0.22
Obese *N* = 8786	1.33 (1.21, 1.46)	0.25 (0.04, 1.66)	0.10
Outcome = odds of using antidepressants			
Underweight *N* = 407	1.68 (1.19, 2.36)	1.25 (0.68, 2.29)	0.91
Normal weight *N* = 22594	1	1	
Overweight *N* = 21434	1.08 (1.00, 1.18)	0.24 (0.05, 1.13)	0.23
Obese *N* = 8786	1.18 (1.06, 1.32)	0.20 (0.03, 1.52)	0.14

N = 53 221 with complete data on any variable included in any analyses.
The multivariable analysis controls for age, gender, smoking, alcohol consumption, physical activity, income and education.
Instrumental variable analysis uses *FTO* (rs9939609) and *MC4R* (rs17782313); *P*-value compares this to the multivariable analysis; this p-value was derived from bootstrapping.

tent with the instrumental variable analyses of BMI and WHR as continuous variables, we found that those who were overweight or obese had reduced odds of outcomes of psychological distress in the instrumental variables analysis.

To understand the different results from the two approaches, one has to consider the underlying assumptions of each method. A causal interpretation of the estimates from multivariable logistic regression assumes that all potential confounders have been measured and adjusted for and that the association is not because of reverse causality. We were able to adjust for smoking, alcohol, physical activity, education and income, which were associated with psychological distress and BMI/WHR in this study. The age- and gender-adjusted associations all attenuated considerably with additional adjustments for these confounders. However, for some of these potential confounders, for example alcohol consumption and self-report of physical activity, there is likely to be measurement error and with better measurement, it is possible that there would have been further attenuation. Residual confounding by unknown confounding factors, such as work control, neighbourhood characteristics and diet, is also possible. Because this is a cross-sectional study, the multivariable results might also be explained by reverse causality, that is, those with psychological distress becoming overweight or obese as a result of symptoms or treatment for their distress.

If we assume that the positive multivariable associations are not because of residual confounding or reverse causality, then several causal mechanisms might explain this association. In a contemporary, Western population overweight or obesity might be related to increased risk of psychological distress via mechanisms involving stigmatization and low self-esteem (i.e. this stigmatization would mediate – rather than confound – an association of greater adiposity with distress). In these populations, slenderness is seen as a marker of beauty and obesity associated with negative attributes, such as laziness. We do not have the possibility to examine this directly in our multivariable (or instrumental variable) analyses. However, if this were a key driver of the positive multivariable association, we might have expected stronger associations in women compared with men and younger individuals compared with older individuals because younger women are likely to be the population subgroup who are more concerned about their appearance and more susceptible to such pressures (consistent with the greater prevalence of eating disorders in younger women). However, we found no consistent evidence of differences by gender or age up to 60 years. Furthermore, we might have expected associations to have a threshold effect being driven largely by greater psychological distress in those who were at the upper end of the distribution. However, the associations were linear across 99% of the distribution of BMI and WHR (i.e. once those who were underweight were removed), with no evidence of a threshold effect in the positive multivariable association.

The instrumental variables are less likely to be affected by measured and unmeasured confounders, because genetic variants are less likely to be associated with lifestyle and socioeconomic characteristics than are nongenetic risk factors [30]. We were able to demonstrate that this was the case for measured confounders in this study population. Because genotype is allocated at conception, reverse causality is not a possible explanation for our instrumental variables results. This approach also provides an estimate of the association of small differences in life time BMI and WHR with psychological distress, because individuals with genotypes related to higher adiposity will have had this influence throughout most of their lives. However, because genotype is related to modest differences in mean BMI/waist (subtle shifts to the right of the whole adiposity distribution), our instrumental variable approach is not a useful way of examining an association that might be explained by stigmatization of those who are at the extreme of the distribution (i.e. those who are obese). Thus, one explanation for the differences in our two analytical approaches would be that greater adiposity has a protective effect against psychological distress via biological mechanism, but in societies where there is marked stigmatization of those who are obese, this stigmatization results in a positive obesity–distress association that outweighs the biological mechanisms that act in the opposite direction. However, as noted above, our gender comparisons, age comparisons and the linear association beyond very low adiposity levels do not support this.

A key underlying instrumental variables assumption for causal analyses is that there is no link between the genetic loci and outcomes other than through their associations with the risk factor of interest (BMI and WHR here). This might be violated by population stratification, pleiotropic effects or canalization [29]. Since this cohort, consisted of white individuals all of Danish descent, population stratification is unlikely. The fact that instrumental variable analyses using

FTO and *MC4R* show expected associations with vascular and metabolic outcomes is further evidence that these are likely to be valid instruments for adiposity [35, 43]. For these outcomes, there is considerably less controversy regarding the existence and direction of a causal effect of adiposity.

If either genotype affected risk of psychological distress (our outcome) directly, rather than only via their association with our risk factors of interest (BMI and WHR), the instrumental variable assumptions would be violated, and the estimator would not be of the causal effect of BMI/WHR on outcome but of the combined effects of this and of the direct genotype-outcome association. It is not possible to test this assumption directly, but we believe it is unlikely to be violated for two reasons. First, neither genotype has been found to be robustly associated with depression in genome-wide association studies. Second, the two genetic variants that we have used as instrumental variables are on different chromosomes and are independently of each other in their associations with BMI and WHR. Whilst the detailed functions of *MC4R* and *FTO* are unknown, they are likely to act on different biological pathways to affect BMI/WHR. Despite this, they produce similar instrumental variable estimates. It would be surprising to get these very similar instrumental variable estimates if they were both acting directly (over and above via their effects on BMI/WHR) on outcomes as it would assume that they produced a direct effect on psychological distress that for each (and despite different associations with BMI/WHR) perfectly matched the indirect effect via BMI/WHR to give the same (biased) instrumental variable estimate [41]. However, we acknowledge that we cannot exclude a direct effect of the genotypes on our outcomes (i.e. other than via their association with BMI/WHR). With additional genetic variants related to adiposity, it would be possible to test this further by combining these in many multiple instrument combinations, if the resulting instrumental variable estimates were similar for each set of combinations, this would further support the results being robust to the instrumental variable assumptions [44]. In addition, it would be valuable to see whether our results replicate in other large studies.

An inverse effect of adiposity on psychological distress would be consistent with biological mechanisms [22–25]. This inverse association is consistent with studies that have reported inverse associations of BMI with hospital admissions for mental health problems and indeed with suicide [9–13].

A key limitation of our study is that we have used soft markers of psychological distress rather than established methods for diagnosing particular psychological disorders. The measures that we have used reflect known symptoms of distress and treatments for depression or anxiety rather than a validated scale/questionnaire. It would be valuable to examine associations of BMI and WHR with depression and other accurately diagnosed diseases using genetic variants as instrumental variables. However, such studies would require very large sample sizes, and we are not aware of any such studies currently available.

In conclusion, our results suggest that the positive association of measurements of adiposity with psychological distress reported in conventional observational studies, and also found here, might be exaggerated by residual confounding or reverse causality. Our instrumental variable analyses suggest that adiposity might protect from psychological distress. Because of the clear adverse effects of adiposity on vascular and metabolic health, demonstrated in multivariable analyses and confirmed using genetic instrumental variables [36, 43], we would not suggest encouraging weight gain as a means of preventing distress. However, our results, if replicated in other large studies and in those that have the potential to examine consistency across a number of genetic instrumental variables that are independent of each other [44], suggest that weight reduction may not be an effective means of preventing psychological distress.

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Disclosure of interest

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Author contributions

Study concept and design: Lawlor, Nordestgaard, Davey Smith. *Acquisition of data*: Nordestgaard, Tybjaerg-Hansen, Zacho, Benn. *Database handling and updating*: Zacho, Benn. *Analysis and interpretation of data*: Lawlor, Harbord, Palmer, Nordestgaard. *Drafting of the manuscript*: Lawlor. *Critical revision of the manuscript for important intellectual content*: Lawlor, Harbord, Palmer, Tybjaerg-Hansen, Zacho, Benn, Timpson, Davey Smith. *Statistical analysis*: Lawlor, Harbord, Palmer. *Obtained funding*: Nordestgaard, Tybjaerg-Hansen. *Administrative, technical, or material support*: Nordestgaard, Zacho, Benn. *Study supervision*: Lawlor, Nordestgaard.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Figure S1. Overlap of outcomes. N = 53221 Danish adults aged 20–99 years.

Table S1. Cohort characteristics. The Copenhagen General Population Study cohort. N = 55,813 eligible (missing data for each variable in final column).

Table S2. Body mass index, waist to hip ratio and covariables by psychological distress outcomes. N = 53,221 with complete data on any variable included in any analyses.

Table S3. Multivariable association of body mass index and waist to hip ratio (as continuous variables) with psychological distress stratified by gender. N = 53,221 (29219 females) with complete data on variable included in any analyses.

Table S4. Multivariable association of body mass index and waist to hip ratio (as continuous variables) with psychological distress stratified by age categories. N = 53,221 with complete data on variable included in any analyses.

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