C-reactive protein levels and body mass index: Elucidating direction of causation through reciprocal Mendelian randomization

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Abstract

Context—The assignment of direction and causality within networks of observational associations is problematic outside randomized control trials and the presence of causal a relationship between body mass index (BMI) and C-reactive protein (CRP) is disputed.
**Objective**—Using reciprocal Mendelian randomization, we aim to assess the direction of causality in relationships between BMI and CRP and to demonstrate this as a promising analytical technique.

**Participants and methods**—The Study was based in a large, cross-sectional European study from Copenhagen, Denmark. Genetic associates of BMI (FTO rs9939609) and circulating CRP (CRPrs3091244) have been used to re-examine observational associations between them.

**Results**—Observational analyses showed strong, positive association between circulating CRP and BMI (change in BMI for a doubling in logCRP of 1.03kg/m² (95%CI 1.00, 1.07), p<0.0001). Analysis using CRPrs3091244 to re-estimate the causal effect of circulating CRP on BMI yielded null effects (change in BMI for a doubling in logCRP of −0.24kg/m² (95%CI −0.58, 0.11), p=0.2). In contrast, analysis using FTOrs9939609 to assess the causal effect of BMI on circulating CRP confirmed observational associations (ratio of geometric means of CRP per standard deviation increase in BMI 1.41(95%CI 1.10, 1.80), p=0.006).

**Conclusions**—Together, these data suggest that the observed association between circulating CRP and measured BMI is likely to be driven by BMI, with CRP being a marker of elevated adiposity. More generally, the method of reciprocal randomization has general applicability in determining direction of causation within inter-correlated networks of metabolic components and methods such as this provide an approach for delivering immediate and clinically applicable information.

**Keywords**

Mendelian randomization; FTO; CRP; hsCRP; BMI

**INTRODUCTION**

The associations between inflammation and obesity-related traits, including impaired insulin resistance, type II diabetes and coronary heart disease, have been investigated extensively in recent years. The classical acute phase protein CRP has been a particular focus of these investigations which have and continue to report associations between circulating CRP levels, obesity and cardiovascular outcomes(1-3). Some prospective studies have suggested that inflammatory markers in general, and CRP in particular, cause the development of elevated adiposity, obesity and diabetes(4-6). Other evidence suggests that obesity is a determinant of inflammatory marker status, including CRP level(1, 7, 8). The direction of causation is difficult to determine outside the realm of experimental trial designs, where exposures may be held constant, because of the highly inter-correlated nature of the factors involved (both exogenous confounders and in the form of bias), the existence of reverse causation and given the inevitable degree of measurement imprecision that may be encountered in such settings(9-11).

Mendelian randomization, the utilization of genetic variants as proxies for particular phenotypic measures, offers a potential approach to assess the direction of association and thus likely causality in observational data. Germ line genetic variants are generally neither associated with confounding factors nor can they be influenced by the outcome measure (i.e. they are not susceptible to reverse causation)(12). Genetic variants related to an intermediate
risk factor of interest (e.g. circulating CRP level) – particularly *cis* variants, which are likely
to reflect effects on gene expression – should produce downstream effects on outcome
phenotypes (e.g. BMI), only if the latter is influenced causally by the intermediate risk factor
in question.

A study with measures of degree of adiposity via BMI, circulating CRP level and genetic
variants related independently to both of these phenotypes allows a particularly clear
assessment of the causal direction of association between BMI and CRP. In this paper we
exploit the properties of variation at the CRP and FTO gene loci in order to perform a
bidirectional Mendelian randomization experiment, which we refer to as a reciprocal
Mendelian randomization. We aimed to elucidate the driving agent behind observational
associations between circulating CRP and BMI, and to illustrate the general potential of this
technique.

**METHODS**

The Copenhagen General Population Study is a cross-sectional study of the Danish general
population initiated in 2003 and still recruiting(13, 14); the aim is to enroll 100,000
participants ascertained using the same methods as those used in the Copenhagen City Heart
Study(15), but with a focus on all multifactorial diseases. At the time of genotyping for the
present study, 37,027 individuals had been included (response rate 45%), however complete
data for BMI, CRP, covariates and genotypes (representing the smallest possible sample size
for analyses within this sample) were available on 21,836 participants. All participants were
white, of Danish descent and were selected based on the national Danish Civil Registration
System to be representative of the adult Copenhagen general population aged 20-80+ years.
Details of data collection procedures have been reported previously(16). For the present
analyses, examination data on height and weight and questionnaire data on age, sex,
smoking, and alcohol consumption, income level and educational level were utilized.
Circulating CRP was assessed and genotyping carried out on extracted DNA.

**Outcome variables**

High sensitivity measurement of circulating CRP (hsCRP) was measured once by high-
sensitivity laser nephelometry (Dade-Behring). Upper and lower limits for circulating values
for CRP were set at 30mg/L and 0.174mg/L respectively. The lowest value was set at the
limits for accurate hsCRP measurement and the highest value was imposed to avoid the
inclusion of those with acute elevation of CRP. Owing to the known highly skewed
distribution of circulating CRP (confirmed in this cohort), this variable was log transformed
prior to analyses to approximate a normal distribution. Where appropriate, results are back
transformed by exponentiation and effects are expressed as ratios of geometric means.

Weight and height were measured once, and BMI was calculated as weight (kg) divided by
height squared (m$^2$). In all regression bar that used for the generation of Figures 1 and 2, we
employed raw measures of BMI. However, to remove the dependence of BMI on sex, age
and height, we generated the measure residual BMI. For this measure BMI was regressed on
sex, age, age squared, log(height) and an age-sex interaction. The residuals from this model
give the difference between an individual’s actual BMI and that expected for their sex, age
and height. For analyses, those with a difference between predicted BMI (independent of variation attributable sex age and height) and observed BMI >20kg/m² were removed (owing to their existence in the extreme tails of the BMI distribution).

Other covariates
Smoking and alcohol consumption were dichotomized and defined as “ever” (ex-smoker or current smoker) versus “never” smokers, and drinkers as those consuming over 36 g alcohol per week. Other possible confounding factors related to social standing and educational attainment were recorded and incorporated into observational analyses. The two responses available for the assessment of these features were years of education completed and earned income at the date of examination. These responses formed the basis of the education and income variables used in further analyses which were coded as: education 0-9yrs, 10-12yrs, >13yrs and annual income <400 000Kr, 400 000-600 000Kr, >600 000Kr (100 000Kr≈13 000Euro≈17 000USD).

Genotyping and the selection of instruments
The ABI PRISM 7900HT Sequence Detection System (Applied Biosystems Inc., Foster City, CA, USA) was used to genotype the FTO locus rs9939609 and the CRP triallelic locus rs3091244 was scored using TaqMan (details available from authors). Genotyping was verified by DNA sequencing in >30 individuals with each genotype. As we performed re-runs twice, >99.9% of all available participants were genotyped.

Using the same population studied here, we have previously shown that the FTO locus rs9939609 is associated with BMI making this polymorphism suitable for a Mendelian randomization study like the present one(17). Likewise, using the same population we previously showed that the CRP triallelic promoter variant rs3091244 was associated with a 67% higher CRP level in plasma for the rarest homozygote versus the most common homozygotes(16).

In resequencing efforts at the CRP locus, Szalai et al(18) assessed approximately 1.2kb of the CRP gene promoter including rs3091244. Electrophoretic mobility shift assay confirmed that this SNP was within an E-box regulatory factor binding site (~394CACTTG-389) supporting hypotheses as to a functional role for this with respect to correlated variation in circulating levels of the CRP protein. We chose to restrict primary analysis to this, the best apparently functional variant. Analyses employed simple genotypic coding of this triallele and categorical analysis in the absence of an assumed genetic model, although in sensitivity analyses (not shown), other combinations were examined as described in Zacho et al(16).

Analyses
All data were gathered in a cross-sectional database summarizing individual characteristics at baseline collection. These data were transferred to Stata 10 (StataCorp LP, 2007) for all analyses. Continuous effects were estimated using linear regression. Mean values for outcome variables by exposure group were estimated from linear regression models allowing for the incorporation of the covariates age and sex (descriptive analyses) and sex,
age, age-squared, age–sex interaction, log(height), smoking, drinking, education and income (BMI/CRP associations).

Instrumental variable methods were used to obtain estimates of the directional effect of BMI on CRP and of CRP on BMI (19-21). The former was performed using FTO (rs9939609) as an instrument for BMI adjusting for sex, age, age-squared, age–sex interaction, log(height), smoking, drinking, education and income. The latter was performed using the SNP CRP (rs3091244) as an instrument for circulating CRP using the same covariates. The inclusion of baseline covariates not associated with instruments was undertaken to maximise the efficiency of instrumental variable regression models and to allow comparison of estimates from observational and instrumental variable analyses. We used the generalized method of moments with robust standard errors to fit the instrumental variable models in the main analyses but checked results using limited information maximum likelihood and two-stage least squares. We compared the instrumental variable estimates to those from ordinary linear regression using the Durbin form of the Durbin-Wu-Hausman statistic (22). We examined F-statistics from the first-stage regressions to evaluate the strength of the instruments. Values greater than ten are often taken to indicate approximate validity of instrumental variable methods (23, 24), while values greater than 30 are sufficient to ensure resulting estimates have under 5% bias and that tests for zero effect conducted at the 5% level have Type I error rates no greater than 10% (25).

RESULTS

There was a strong age-adjusted observational association between CRP and BMI among men and women as seen in Table 1. CRP levels were higher in women than men across the BMI distribution and across a large proportion of the BMI distribution the association between CRP and BMI (as for residual BMI) was approximately linear (Supplementary Figure S1). In fully adjusted analyses, the change in logCRP for a standard deviation increase in BMI can be summarized by a ratio of geometric means of 1.46 (95% CI 1.45, 1.48), which approximates to a 0.71 mg/L increase in circulating CRP. Sex-stratified analyses of this relationship showed a ratio of geometric means of 1.49 (95% CI 1.47, 1.52), ~0.76 mg/L change in women, and 1.42 (1.39, 1.45), ~0.65 mg/L change in men (p\text{het} between men and women <0.0001). Conversely, if BMI was treated as the outcome, in fully adjusted analysis there was a 1.06 (95% CI 1.02, 1.09) kg/m$^2$ increase in BMI for a doubling in logCRP. In sex-stratified analyses, the same association was 1.26 (95% CI 1.21, 1.31) kg/m$^2$ in women and 0.82 (0.77, 0.86) kg/m$^2$ men (p\text{het} <0.0001).

In age and sex adjusted analyses, CRP levels were strongly associated with sex, age, education, smoking, income and alcohol consumption (Table 2). BMI was associated with all of these, except for smoking (Table 3). After stratification of these analyses by sex, largely consistent patterns were found. Out of these variables, the only relationships not shown to have strong evidence for association were those between the proportion with low income by quintile of CRP (p=0.9, males only) and the proportion with high income by quintile of BMI (p=0.04, males only) (Supplementary Table S2-S5). All other features showed evidence for association (p<0.005). Furthermore, adjusting the associations between
BMI and CRP for sex, age, age$^2$, age$^X$sex interaction, logheight and the confounders smoking, drinking, education and income had little effect on estimates (Table 1).

$FTO$(rs9939609) genotype was associated with BMI in a manner expected given previous work in this population(17) (Table 4), a finding that was similar in males and in females and in different age groups (not shown). $FTO$ variation was also associated with CRP levels, with genotypes associated with higher BMI being associated with higher CRP levels (ratio of geometric means from additive model 1.03(95%CI 1.01, 1.05), p=0.003). Variation at $CRP$(rs3091244) was strongly associated with CRP levels, again in the expected manner(16) (ratio of geometric means from additive model 1.11(95%CI 1.10, 1.13), p<0.0001) (Table 4). As for $FTO$(rs9939609), the effect were similar in both sexes and in different age groups (not shown). There was, however, no association between $CRP$(rs3091244) genotype and BMI. There was no strong evidence of association between either $FTO$(rs9939609) or $CRP$(rs3091244) and confounding factors (Supplementary Table S1).

The joint associations of $FTO$ genotype and BMI, and $FTO$ genotype and CRP were used within an instrumental variable analysis to derive an estimate of the causal effect of BMI as an exposure on CRP as an outcome. In this instrumental variable analysis, the ratio of geometric means of circulating CRP per standard deviation increase in BMI was 1.41(95%CI 1.10, 1.80), p=0.006. In a test of equivalence between associations derived from observational analyses and from instrumental variable analysis, there was no evidence for difference (p$^{diff} =0.8$). However, when $CRP$ genotypes were used as an instrument for circulating CRP levels to estimate the causal effect of CRP level on BMI, observational associations were not corroborated. For a doubling in logCRP, the average change in BMI was estimated to be $-0.24$kg/m$^2$(95%CI $-0.58$, 0.11), p=0.2. In this case, a comparison of observational and instrumental variable estimates showed strong contrast (p$^{diff} <0.0001$). These results are summarised in Table 5. Instrumental variable analyses excluding baseline covariates show no substantive departures from reported effects (data not shown).

Figures 1 and 2 illustrate these findings graphically and note the contrast in effect estimates derived from instrumental variable analyses using $FTO$ and $CRP$ variation as instruments for BMI and CRP respectively. These figures employ the measure residual BMI to test for consistency in results when using a measure of BMI independent of sex, age and height.

These instrumental variable analyses were run separately for both sexes and in three different age groups, and generally consistent findings emerged (Supplementary Table S6/ S7). In the case of age, where observational associations between BMI and circulating CRP diminish with age, instrumental variable derived associations between BMI and CRP were similar across age strata.

**DISCUSSION**

We employed a reciprocal Mendelian randomization design to explore relationships between circulating CRP level and BMI. Not only was variation at $CRP$ employed to evaluate whether CRP has a causal effect on BMI, but simultaneously, variation at $FTO$ was used to evaluate whether BMI has a causal effect on CRP. This scheme is summarized in Figure 3.
and exploits the availability of two independent instruments that give non-confounded estimates of causal effect. Overall, the use of variation at the CRP and FTO loci in reciprocal tests for causal association between circulating CRP and BMI (in both basic genetic association and instrumental variable analyses) showed marked contrast and provided evidence that adiposity causally influences circulating CRP levels and not vice versa.

Previous work has noted the consistent relationship between BMI and markers of chronic inflammation, including circulating CRP(21, 26-31). However, there have been questions regarding the causal nature of relationships between these measures and continued discussion of the appropriate interpretation of high sensitivity CRP measures in a clinical setting(32). While the measurement of chronic elevation in circulating CRP has potential with respect to the refinement of prediction models for cardiovascular disease and endpoint events(33), this is not dependent on causality (despite published assertions regarding this possibility)(5, 34).

Work from studies such as the JUPITER trial(35) and from apparently independent assessment of CRP action(36, 37) has continued to suggest that the role of CRP may be more than a marker of both BMI-derived and non-BMI related cardiovascular risk. Difficulty clearly exists as to identifying a causal role for CRP due in part to its confounded nature(38) and the potential for reverse causality(21). In the absence of conventional randomization studies reciprocal Mendelian randomization offers valuable insight. We consider the example presented – where there is widespread but not universal consensus that the direction of causality runs from adiposity to CRP, not vice versa – as an illustration of a potentially highly valuable research strategy. Indeed in a number of other situations it is already possible to identify genetic variants related to levels of associated pairs of factors where the direction of causality requires elucidation and the increasing success of genome wide association studies in identifying common variants related to intermediate phenotypes(39) will render this approach more generally applicable.

BMI as a causal agent

Available literature has confirmed the association between BMI and CRP and pointed towards BMI as a major contribution to observed variation in CRP levels in differing populations(7, 40-42). With this has also come recognition that the adipocyte itself is a key expressor of inflammatory molecules and that in situations of increased adiposity, levels of CRP expression are elevated(1, 8, 40, 42). This is not restricted to CRP; other inflammatory cytokines show elevated patterns of expression in situations of increased adiposity(1, 43). In addition to these biological lines of evidence suggesting that there is a direct link between increased levels of adiposity and changes in chronic inflammatory profiles, epidemiological evidence has shown similar patterns. Consistently, the adjustment of observational associations between circulating CRP and components of the metabolic syndrome for adiposity has led to an attenuation of these relationships. This suggests that, whilst CRP is indeed marking the events leading to metabolic disturbance, it may not be driving them directly or independently(1, 44). The dilemma with observational data, however, is that such statistical adjustments are highly dependent on the measurement characteristics of the
confounders, and rely on the confounders being known and measurable(9). Lastly, available evidence about the impact of weight loss has on circulating levels of CRP again favour the findings presented here. Weight loss interventions have been shown to decrease levels of circulating CRP and to improve the metabolic profile of those concerned(45). Furthermore, investigation into the effects of weight reduction on the expression of inflammation-regulated loci has again shown that adiposity appears to be important in influencing levels of inflammatory proteins(46).

Limitations to Mendelian Randomisation

There are several potential limitations to the application of Mendelian randomization, which have been discussed at length previously(20, 47). Firstly, the biological consequences of variation at the FTO locus and the mechanism of the observed association of this with fat mass are still unclear. Several studies exists which point to a role for this locus in energy regulation and hypothalamically regulated patterns of appetite(48-52); however the possibility of complicating pleiotropy in the action of FTO variation cannot be completely ruled out. In this case utilizing multiple instruments – i.e. multiple independent genetic variants that relate to an intermediate phenotype, such as BMI – can help strengthen causal inference, as pleiotropic effects are unlikely to influence the effects of each instrument in the same manner(20). Secondly, the possibility of developmental plasticity altering the impact of chronic changes of metabolism delivered by heritable change (otherwise termed canalization(53)) cannot be taken into account though may influence the interpretation of findings.

Ultimately such findings need to be interpreted against the background of other evidence on specific associations, but the reciprocal Mendelian randomization approach we advance here can provide powerful support to efforts at elucidating the direction of causal pathways. Through this, it offers to contribute to clinical and population-level efforts to improve health through modifying causal pathways leading to disease.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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References

1. Yudkin JS, Stehouwer CD, Emeis JJ, Coppack SW. C-reactive protein in healthy subjects: associations with obesity, insulin resistance, and endothelial dysfunction: a potential role for cytokines originating from adipose tissue? Arteriosclerosis, Thrombosis & Vascular Biology. 1999; 19:972–978.

2. Collaboration TERF. C-reactive protein concentration and risk of coronary disease, stroke, and mortality: an individual participant meta-analysis. The Lancet. 2010; 375:132–140.

3. Dandona P, Aljada A, Bandyopadhyay A. Inflammation: the link between insulin resistance, obesity and diabetes. Trends in Immunology. 2004; 25:4–7. [PubMed: 14698276]

4. Dandona P, Weinstock R, Thusu K, Abdel-Rahman E, Aljada A, Wadden T. Tumor necrosis factor-alpha in sera of obese patients: fall with weight loss. Journal of Clinical Endocrinology & Metabolism. 1998; 83:2907–2910. [PubMed: 9709967]

5. Bochud M, Fabienne M, Marques-Vidal P, Vollenweider P, Beckmann JS, Mooser V, et al. Association between C-reactive protein and adiposity in women. Journal of Endocrinology and Metabolism. 2009

6. Schmidt MI, Duncan BB, Sharrett AR, Lindberg G, Savage PJ, Offenbacher S, et al. Markers of inflammation and prediction of diabetes mellitus in adults (Atherosclerosis Risk in Communities study): a cohort study. Lancet. 1999; 353:1649–1652. [PubMed: 10335783]

7. Greenfield JR, Samaras K, Jenkins AB, Kelly PJ, Spector TD, Gallimore JR, et al. Obesity is an important determinant of baseline serum C-reactive protein concentration in monozygotic twins, independent of genetic influences. Circulation. 2004; 109:3022–3028. [PubMed: 15184288]

8. Calabro P, Chang DW, Willerson JT, Yeh ETH. Release of C-reactive protein in response to inflammatory cytokines by human adipocytes: linking obesity to vascular inflammation. Journal of the American College of Cardiology. 2005; 46:1112–1113. [PubMed: 16168299]

9. Phillips AN, Smith GD. How independent are “independent” effects? Relative risk estimation when correlated exposures are measured imprecisely. Journal of Clinical Epidemiology. 1991; 44:1223–1231. [PubMed: 1941017]

10. Davey Smith G. Reflections on the limitations to epidemiology. Journal of Clinical Epidemiology. 2001; 54:325–331. [PubMed: 11297881]

11. Davey Smith G, Ebrahim S. Mendelian Randomisation: prospects, potentials and limitations. International Journal of Epidemiology. 2004; 33:30–42.

12. 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. [PubMed: 18076282]

13. Nordestgaard BG, Benn M, Schnohr P, Tybjaerg-Hansen A. Nonfasting triglycerides and risk of myocardial infarction, ischemic heart disease, and death in men and women. JAMA. 2007; 298:299–308. [PubMed: 17635890]

14. Frikke-Schmidt R, Nordestgaard BG, Steine MCA, Sethi AA, Remaley AT, Schnohr P, et al. Association of loss-of-function mutations in the ABCA1 gene with high-density lipoprotein cholesterol levels and risk of ischemic heart disease. JAMA. 2008; 299:2524–2532. [PubMed: 18523221]

15. Schnohr P, Jensen JS, Scharling H, Nordestgaard BG. Coronary heart disease risk factors ranked by importance for the individual and community. A 21 year follow-up of 12 000 men and women from The Copenhagen City Heart Study. European Heart Journal. 2002; 23:620–626. [PubMed: 11969276]

16. Zacho J, Tybjaerg-Hansen A, Jensen JS, Grande P, Sillensen H, Nordestgaard BG. Genetically elevated C-reactive protein and ischaemic vascular disease. New England Journal of Medicine. 2008; 359:1897–1908. [PubMed: 18971492]
17. Timpson NJ, Harbord R, Davey Smith G, Zacho J, Tybjaerg-Hansen A, Nordestgaard BG. Does greater adiposity increase blood pressure and hypertension risk? Mendelian randomisation using FTO/MC4R genotype. Hypertension. 2009; 54:84–90. [PubMed: 19470880]

18. Szalai AJ, Wu J, Lange EM, McCrory MA, Langefeld CD, Williams A, et al. Single-nucleotide polymorphisms in the C-reactive protein (CRP) gene promoter that affect transcription factor binding, alter transcriptional activity, and associate with differences in baseline serum CRP level. Journal of Molecular Medicine. 2005; 83:440–447. [PubMed: 15778807]

19. Greenland S. An introduction to instrumental variables for epidemiologists. International Jounal of Epidemiology. 2000; 29:722–729.

20. Lawlor DA, Harbord RM, Sterne JAC, Timpson NJ, Davey Smith G. Mendelian randomization: Using genes as instruments for making causal inferences in epidemiology. Statistics in Medicine. 2008; 27:1133–1163. [PubMed: 17886233]

21. Timpson NJ, Lawlor DA, Harbord R, Gaunt TR, Day IN, Palmer LJ, et al. C-reactive protein and metabolic syndrome: Mendelian randomisation suggests associations are non-causal. The Lancet. 2005; 366:1954–1959.

22. Durbin J. Errors in variables. Review of the International Statistics Institute. 1954; 22:23–32.

23. Stock JH, Wright JH, Yogo M. A survey of weak instruments and weak identification in generalized method of moments. Journal of Business & Economic Statistics. 2002; 20:518–529.

24. Staiger D, Stock JH. Instrumental Variables Regression with Weak Instruments. Econometrica: Journal of the Econometric Society. 1997; 65:557–586.

25. Stock, JH; Yogo, M. Testing for Weak Instruments in Linear IV Regression. In: Andrews, DWK.; Stock, JH., editors. Identification and Inference for Econometric Models: Essays in Honor of Thomas Rothenberg. Cambridge University Press; Cambridge: 2005. p. 80-108.

26. Frohlich M, Imhof A, Berg G, Hutchinson WL, Pepys MB, Boeijing H, et al. Association between C-reactive protein and features of the metabolic syndrome: a population based study. Diabetes Care. 2000; 23:1835–1839. [PubMed: 11128362]

27. Visser M, Bouter LM, McQuillan GM, Wener MH, Harris TB. Low-grade systemic inflammation in overweight children. Pediatrics. 2001; 107:E13. [PubMed: 11134477]

28. Fantuzzi G, Fantuzzi G. Adipose tissue, adipokines, and inflammation. J Allergy Clin Immunol. 2005; 115:911–919. quiz 920. [PubMed: 15867843]

29. Lowe GD, Rumley A, Wannamethee SG. Hemostatic abnormalities associated with obesity and the metabolic syndrome. Journal of Thrombosis and Haemostasis. 2005; 3:1076–1078.

30. Ridker PM, Buring JE, Cook NR, Rifai N. C-reactive protein, the metabolic syndrome, and risk of incident cardiovascular events: an 8-year follow-up of 14 719 initially healthy American women. Circulation. 2003; 107:391–397. [PubMed: 12551861]

31. Rutter MK, Meigs JB, Sullivan LM, D’Agostino RB Sr, Wilson PW. C-reactive protein, the metabolic syndrome, and prediction of cardiovascular events in the Framingham Offspring Study. Circulation. 2004; 110:380–385. [PubMed: 15262834]

32. Clinical Chemistry. 2009; 55(2) Special Issue - CRP.

33. Hingorani AD, Shah T, Casas JP, Humphries SE, Talmud PJ, Hingorani AD, et al. C-reactive protein and coronary heart disease: predictive test or therapeutic target? Clinical Chemistry. 2009; 55:239–255. [PubMed: 19114670]

34. Devaraj S, Singh U, Jialal I, Devaraj S, Singh U, Jialal I. The evolving role of C-reactive protein in atherothrombosis. Clinical Chemistry. 2009; 55:229–238. [PubMed: 19095731]

35. Ridker PM, Danielson E, Fonseca FA, Genest J, Gotto AM Jr, Kastelein JJ, et al. Rosuvastatin to prevent vascular events in men and women with elevated C-reactive protein. New England Journal of Medicine. 2008; 359:2195–2207. [PubMed: 18997196]

36. Ridker PM, Rifai N, Pfeffer MA, Sacks F, Braunwald E. Long-term effects of pravastatin on plasma concentration of C-reactive protein. The Cholesterol and Recurrent Events (CARE) Investigators. Circulation. 1999; 100:230–235. [PubMed: 10411845]

37. Scirica BM, Cannon CP, Sabatine MS, Jarolim P, Sloane S, Rifai N, et al. Concentrations of C-reactive protein and B-type natriuretic peptide 30 days after acute coronary syndromes independently predict hospitalization for heart failure and cardiovascular death. Clinical Chemistry. 2009; 55:265–273. [PubMed: 19074516]
38. Ruckerl R, Peters A, Khuseyinova N, Andreani M, Koenig W, Meisinger C, et al. Determinants of the acute-phase protein C-reactive protein in myocardial infarction survivors: the role of comorbidities and environmental factors. Clinical Chemistry. 2009; 55:322–335. [PubMed: 19095729]

39. Hindorff LA, Sethupathy P, Junkins HA, Ramos EM, Mehta JP, Collins FS, et al. Potential etiologic and functional implications of genome-wide association loci for human diseases and traits. Proceedings of the National Academy of Sciences. 2009; 106:9362–9367.

40. Casas JP, Shah T, Hingorani AD, Danesh J, Pepys MB. C-reactive protein and coronary heart disease: a critical review. Journal of Internal Medicine. 2008; 264:295–314. [PubMed: 18823504]

41. Park HS, Park JY, Yu R. Relationship of obesity and visceral adiposity with serum concentrations of CRP, TNF-alpha and IL-6. Diabetes Research & Clinical Practice. 2005; 69:29–35. [PubMed: 15955385]

42. Aronson D, Bartha P, Zinder O, Kerner A, Markiewicz W, Avizohar O, et al. Obesity is the major determinant of elevated C-reactive protein in subjects with the metabolic syndrome. International Journal of Obesity & Related Metabolic Disorders: Journal of the International Association for the Study of Obesity. 2004; 28:674–679.

43. Hotamisligil GS, Arner P, Caro JF, Atkinson RL, Spiegelman BM. Increased adipose tissue expression of tumor necrosis factor-alpha in human obesity and insulin resistance. Journal of Clinical Investigation. 1995; 95:2409–2415. [PubMed: 7738205]

44. Kahn SE, Zinman B, Haffner SM, O'Neill MC, Kravitz BG, Yu D, et al. Obesity is a major determinant of the association of C-reactive protein levels and the metabolic syndrome in type 2 diabetes. Diabetes. 2006; 55:2357–2364. [PubMed: 16873701]

45. Selvin E, Paynter NP, Erlinger TP. The effect of weight loss on C-reactive protein: a systematic review. Archives of Internal Medicine. 2007; 167:31–39. [PubMed: 17210875]

46. Clement K, Viguerie N, Poitou C, Carette C, Pelloux V, Curat CA, et al. Weight loss regulates inflammation-related genes in white adipose tissue of obese subjects. Faseb J. 2004; 18:1657–1669. [PubMed: 15522911]

47. Davey Smith G, Ebrahim S. ‘Mendelian Randomisation’: can genetic epidemiology contribute to understanding environmental determinants of disease? International Journal of Epidemiology. 2003; 32:1–22.

48. Timpson NJ, Emmett P, Frayling TM, Rogers I, Hattersley AT, McCarthy MI, et al. The FTO/obesity associated locus and dietary intake in children. American Journal of Clinical Nutrition. 2008; 88:971–978. [PubMed: 18842783]

49. Fischer J, Koch L, Emmerling C, Vierkotten J, Peters T, Bruning JC, et al. Inactivation of the Fto gene protects from obesity. Nature. 2009; 458:894–898. [PubMed: 19234441]

50. Stratigopoulos G, Padilla SL, LeDuc CA, Watson E, Hattersley AT, McCarthy MI, et al. Regulation of Fto/Ftm gene expression in mice and humans. AJP - Regulatory, Integrative and Comparative Physiology. 2008; 294:R1185–1196.

51. Cecil JE, Tavendale R, Watt P, Hetherington MM, Palmer CNA. An obesity-associated FTO gene variant and increased energy intake in children. New England Journal of Medicine. 2008; 359:2558–2566. [PubMed: 19073975]

52. Gerken T, Girard CA, Tung YL, Webby CJ, Saudek V, Hewitson KS, et al. The obesity-associated FTO gene encodes a 2-oxoglutarate-dependent nucleic acid demethylase. Science. 2007; 318:1469–1472. [PubMed: 17991826]

53. Waddington CH. Canalisation of development and the inheritance of acquired characters. Nature. 1942; 150:563–565.
Figure 1.
Comparison of linear relationships between circulating CRP and residual BMI observationally and when estimated employing FTO loci as an instrument for residual BMI. X and Y axes represent residual BMI and CRP respectively. Light blue points represent a scatter plot of the correlation between circulating CRP and residual BMI. Grey areas represents 95% confidence regions around instrumental variables estimates. Black area represents 95% confidence regions around simple linear regression estimates. (The 50 individuals with extreme residual BMI over 20 kg/m² are not shown on the plot but were included in the analyses that gave the fitted lines and confidence regions.)
Figure 2.
Comparison of linear relationships between residual BMI and circulating CRP observationally and when estimated employing the CRP locus rs3091244 as an instrument for log transformed CRP.
X and Y axes represent CRP and residual BMI respectively.
Light blue points represent a scatter plot of the correlation between circulating CRP and residual BMI.
Grey areas represent 95% confidence regions around instrumental variables estimates.
Black area represents 95% confidence regions around simple linear regression estimates.
(The 50 individuals with extreme residual BMI over 20 kg/m² are not shown on the plot but were included in the analyses that gave the fitted lines and confidence regions.)
Figure 3.
Graphical representation of the reciprocal Mendelian randomization framework used in main analyses.
Dotted line represents the unknown direction of relationship between circulating CRP and BMI.
Relationships (i) and (ii) denote the informative associations between CRP genotypes, FTO genotypes and circulating CRP and body mass index.
Single-headed arrows represent the known (and assumed causal and largely non-confounded) relationships between variation at the CRP and FTO loci and circulating CRP and body mass index respectively.
### Table 1: Age adjusted means for CRP and BMI levels by decile of BMI

| BMI/CRP by BMI decile | N  | 1     | 2     | 3     | 4     | 5     | 6     | 7     | 8     | 9     | 10    | Beta  | Beta' |
|-----------------------|----|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|
| BMI                   | 23073 | 20.20 (20.12, 20.28) | 20.15 (20.04, 20.26) | 20.15 (20.04, 20.26) | 20.15 (20.04, 20.26) | 20.15 (20.04, 20.26) | 20.15 (20.04, 20.26) | 20.15 (20.04, 20.26) | 20.15 (20.04, 20.26) | 20.15 (20.04, 20.26) | 20.15 (20.04, 20.26) | 20.15 (20.04, 20.26) | 1.134 (1.129, 1.139) | 1.137 (1.132, 1.142) |
| CRP                   | 22208 | 0.47 (0.44, 0.51) | 0.52 (0.49, 0.56) | 0.54 (0.51, 0.58) | 0.61 (0.57, 0.65) | 0.63 (0.59, 0.67) | 0.75 (0.70, 0.80) | 0.80 (0.75, 0.86) | 0.81 (0.75, 0.86) | 1.09 (1.02, 1.17) | 1.63 (1.52, 1.75) | |
| BMI                   | 12153 | 20.15 (20.04, 20.26) | 22.20 (22.12, 22.28) | 23.39 (23.31, 23.47) | 24.40 (24.31, 24.48) | 25.55 (25.27, 25.44) | 26.34 (26.26, 26.43) | 27.43 (27.35, 27.52) | 28.72 (28.64, 28.81) | 30.55 (30.47, 30.64) | 35.26 (35.16, 35.34) | |
| CRP                   | 11656 | 0.56 (0.53, 0.63) | 0.65 (0.60, 0.71) | 0.67 (0.61, 0.71) | 0.79 (0.72, 0.87) | 0.80 (0.74, 0.84) | 1.02 (0.92, 1.12) | 1.00 (0.90, 1.20) | 1.27 (1.15, 1.40) | 1.62 (1.47, 1.78) | 2.32 (2.11, 2.55) | |

Means (95% CI) presented above are age adjusted and for logCRP are expressed as geometric means.

- “f” and “m” represent female and male specific values respectively.
- “Beta” represents linear regression (95% CI) derived beta coefficient expressed as a ratio of geometric means for the association between logCRP and decile of BMI.
- Beta’ indicates adjustment for sex, age, age-squared, age–sex interaction, log(height), smoking, drinking, education and income.
Table 2

Age and sex adjusted relationships between confounders and quintiles of CRP

| Confounder                  | CRP Quintile |       |       |       |       | p    |
|-----------------------------|--------------|-------|-------|-------|-------|------|
|                             | 1            | 2     | 3     | 4     | 5     |      |
| Mean age ¥ (n=22304)        | 53.4 (53.0, 53.7) | 57.0 (56.6, 57.4) | 59.1 (58.7, 59.4) | 59.9 (59.5, 60.3) | 60.9 (60.5, 61.3) | <0.0001 |
| % Male ¥# (n=22304)         | 49.6 (48.4, 50.7) | 48.7 (47.9, 49.5) | 47.6 (46.9, 48.3) | 46.3 (45.5, 47.1) | 45.1 (44.0, 46.2) | <0.0001 |
| % Ever smokers ¥ (n=22304)  | 55.4 (54.2, 56.5) | 59.3 (58.6, 60.2) | 62.6 (62.0, 63.3) | 65.5 (64.7, 66.2) | 68.0 (66.9, 69.0) | <0.0001 |
| % Ever drinkers ¥ (n=22304) | 74.6 (73.6, 75.6) | 73.9 (73.2, 74.6) | 72.1 (71.5, 72.7) | 69.9 (69.2, 70.7) | 66.9 (65.8, 68.0) | <0.0001 |
| % Low Income ¥ (n=22017)    | 35.2 (34.0, 36.4) | 45.9 (45.0, 46.8) | 54.0 (53.2, 54.8) | 59.3 (58.4, 60.2) | 65.2 (64.0, 66.4) | <0.0001 |
| % High income ¥ (n=22017)   | 12.1 (11.3, 12.9) | 8.8 (8.4, 9.3)    | 6.9 (6.5, 7.3)    | 5.7 (5.3, 6.1)    | 4.6 (4.2, 5.0)    | <0.0001 |
| % Low education ¥ (n=22211) | 23.7 (22.7, 24.8) | 32.5 (31.6, 33.3) | 39.7 (38.9, 40.4) | 45.0 (44.1, 45.9) | 5.1 (4.9, 5.2)    | <0.0001 |
| % High education ¥ (n=22211)| 13.5 (12.7, 14.4) | 10.0 (9.5, 10.5)  | 8.1 (7.7, 8.5)    | 7.1 (6.7, 7.6)    | 6.2 (5.7, 6.7)    | <0.0001 |

¥ indicates age and sex adjusted proportion (95%CI) of confounder by quintile of CRP.
¥# indicates sex adjusted (95%CI) of confounder by quintile of CRP.
# indicates age adjusted proportion (95%CI) of confounder by quintile of CRP.

Smoking and drinking are binary variables and are coded as: smoking ever/never smoked, drinking >36g/wk.

High/Low education and income are represented by upper and lower groups of the tripartite variables education = 0-9yrs, 10-12yrs, >13yrs and income = <400 000Kr, 400 000-600 000Kr, >600 000Kr.
### Table 3

Age and sex adjusted relationships between confounders and quintiles of BMI

| Confounder          | BMI Quintile | 1            | 2            | 3            | 4            | 5            | p            |
|---------------------|--------------|--------------|--------------|--------------|--------------|--------------|--------------|
| Mean age¥ (n=23073) | 54.9 (54.5, 55.3) | 56.7 (56.3, 57.1) | 58.4 (58.0, 58.7) | 59.8 (59.4, 60.2) | 59.2 (58.8, 59.5) | <0.0001      |
| % Male# (n=23073)   | 36.2 (35.1, 37.3) | 41.6 (40.9, 42.4) | 47.3 (46.6, 47.9) | 53.0 (52.2, 53.8) | 58.5 (57.4, 59.6) | <0.0001      |
| % Ever smokers† (n=23073) | 60.0 (58.9, 61.1) | 61.5 (60.7, 62.3) | 62.5 (61.9, 63.2) | 63.0 (62.2, 63.8) | 61.9 (60.8, 63.0) | 0.3          |
| % Ever drinkers† (n=23073) | 73.3 (72.3, 74.3) | 73.8 (73.1, 74.5) | 73.3 (72.6, 73.9) | 71.4 (70.7, 72.1) | 66.4 (65.3, 67.5) | <0.0001      |
| % Low Income‡ (n=22780) | 45.2 (43.9, 46.4) | 47.8 (46.9, 48.8) | 51.3 (50.5, 52.0) | 55.2 (54.3, 56.1) | 56.4 (55.2, 57.7) | <0.0001      |
| % High income‡ (n=22780) | 10.0 (9.3, 10.8) | 8.8 (8.3, 9.3) | 7.6 (7.2, 7.9) | 6.4 (6.0, 6.8) | 5.7 (5.2, 6.2) | <0.0001      |
| % Low education‡ (n=22979) | 23.0 (22.0, 24.0) | 30.0 (29.2, 30.8) | 37.8 (37.1, 38.5) | 45.7 (44.8, 46.6) | 49.5 (48.2, 50.8) | <0.0001      |
| % High education‡ (n=22979) | 13.7 (12.9, 14.6) | 11.0 (10.5, 11.5) | 8.7 (8.3, 9.1) | 6.9 (6.5, 7.3) | 6.1 (5.7, 6.7) | <0.0001      |

† indicates age and sex adjusted proportion (95%CI) of confounder by quintile of BMI.
¥ indicates sex adjusted proportion (95%CI) of confounder by quintile of BMI.
# indicates age adjusted proportion (95%CI) of confounder by quintile of BMI.

Smoking and drinking are binary variables and are coded as: smoking ever/never smoked, drinking >36g/wk.

High/Low education and income are represented by upper and lower groups of the tripartite variables education = 0-9yrs, 10-12yrs, >13yrs and income = <400 000Kr, 400 000-600 000Kr, >600 000Kr.
Table 4

Relationships between genotypic variation and BMI and circulating CRP.

|                | FTO(rs939609) |                | CRP(rs3091244) |
|----------------|---------------|----------------|----------------|
|                | TT            | AT             | AA             | CC       | CT       | TT       | CA       | AT       | AA       | Per allele effect | p            |
| BMI            | 26.07 (25.98, 26.17) | 26.37 (26.29, 26.45) | 26.73 (26.59, 26.87) | 26.32 (26.23, 26.41) | 26.36 (26.27, 26.44) | 26.24 (26.07, 26.42) | 26.25 (26.02, 26.47) | 26.29 (25.98, 26.61) | 27.15 (26.02, 28.28) | −0.01 (−0.06, 0.04) | 0.7          |
| CRP            | 1.51 (1.48, 1.55)  | 1.55 (1.52, 1.58) | 1.61 (1.56, 1.67) | 1.37 (1.34, 1.40)  | 1.61 (1.57, 1.64)  | 1.82 (1.74, 1.90)  | 1.71 (1.62, 1.81)  | 2.11 (1.95, 2.28)  | 2.56 (1.95, 3.37)  | 1.11 (1.10, 1.13)  | <0.0001      |

Means (95%CI) by genotypes with linear regression derived, per allele effect estimates (assuming additivity).

CRP was log transformed for analyses hence geometric means are presented by genotype and a ratios of geometric for effect estimates.
Table 5

Observational and instrumental variable derived relationships between BMI and circulating CRP.

| Outcome/explanatory variable | Observational | Instrumental variable | P IV | P diff | F first |
|------------------------------|---------------|-----------------------|------|--------|---------|
| CRP/BMI                      | 1.46 (1.44, 1.48) | 1.41 (1.10, 1.80) | 0.006 | 0.8    | 31.1    |
| BMI/CRP                      | 1.03 (1.00, 1.07) | −0.24 (−0.58, 0.11) | 0.2  | <0.0001 | 57.3    |

Observational analysis effects (95%CI) derived from linear regression adjusted for sex, age, age-squared, age–sex interaction, log(height), smoking, drinking, education and income. CRP is log transformed for analyses above and effects on CRP are shown as ratios of geometric means for a standard deviation increase in BMI. BMI effects are expressed as kg/m^2 for a doubling in logCRP.

Instrumental variable derived estimates of the same effects include the same covariates.

P IV is the p-value from a test that the instrumental variable estimate is equal to the null.

P diff is the p-value from a test for difference between the observational and instrumental variable estimates.

F first is the first stage F-statistic from instrumental variable analysis.