Association between C-Reactive Protein and Adiposity in Women

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Context: The link between C-reactive protein (CRP) and adiposity deserves to be further explored, considering the controversial diabetogenic role of CRP.

Objective: We explored the potential causal role of CRP on measures of adiposity.

Design: We used a Mendelian randomization approach with the *CRP* and *LEPR* genes as instrumental variables in a cross-sectional Caucasian population-based study comprising 2526 men and 2836 women. Adiposity was measured using body mass index (BMI), fat and lean mass estimated by bioelectrical impedance, and waist circumference.

Results: Log-transformed CRP explained by the *rs7553007* single-nucleotide polymorphism tagging the *CRP* gene was significantly associated with BMI [regression coefficient: 1.22 (0.18; 2.25), $P = 0.02$] and fat mass [2.67 (0.65; 4.68), $P = 0.01$] but not with lean mass in women, whereas no association was found in men. Log-transformed *CRP* explained by the *rs1805096 LEPR* single-nucleotide polymorphism was also positively associated, although not significantly, with BMI or fat mass. The combined *CRP-LEPR* instrument explained 2.24 and 0.77% of CRP variance in women and men, respectively. Log-transformed CRP explained by this combined instrument was significantly associated with BMI [0.98 (0.32; 1.63), $P = 0.004$], fat mass [2.07 (0.79; 3.34), $P = 0.001$], and waist [2.09 (0.39; 3.78), $P = 0.01$] in women but not men.

Conclusion: Our data suggest that CRP is causally and positively related to BMI in women and that this is mainly due to fat mass. Results on the combined *CRP-LEPR* instrument suggest that leptin may play a role in the causal association between CRP and adiposity in women. Results in men were not significant. **(*J Clin Endocrinol Metab* 94: 3969–3977, 2009)**

Raised C-reactive protein (CRP), a sensitive but nonspecific marker of inflammation, is associated with increased cardiovascular disease and mortality (1, 2). Plasma CRP predicts the incidence of metabolic syndrome and type 2 diabetes mellitus (3–6). Genetic variants in the *CRP* gene that are associated with higher CRP levels have been shown to predict incident type 2 diabetes mellitus in one study (7), which suggests that CRP might be causally involved in the pathogenesis of type 2 diabetes, a hallmark of which is abdominal obesity. This was however not confirmed in a recent study by Brunner *et al.* (8). Despite the usefulness of measuring plasma CRP levels for cardiovascular risk stratification (9, 10) and the recent proposal to include CRP as one of the clinical criteria for the metabolic syndrome (11), the precise pathophysiological role of CRP in hu-

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Abbreviations: BMI, Body mass index; CRP, C-reactive protein; *LEPR*, leptin receptor gene; OLS, ordinary least square; 2SLS, two-stage least squares; SNP, single-nucleotide polymorphism.

mans remains unclear. In particular, the link between CRP and adiposity deserves to be further explored.

We used Mendelian randomization to explore the potential causal association of CRP with adiposity, estimated using body mass index (BMI), fat mass, and waist circumference in a large population-based study comprising 5362 Caucasian individuals. The purpose of the Mendelian randomization approach is to allow estimation of causal effects, despite confounding factors (12). CRP variation is known to be heritable (13) and variants located within or around the *CRP* gene explain part of this genetic variance (14, 15). The *CRP* gene represents the best candidate gene for a Mendelian randomization study using plasma CRP levels. Recently other genes have been associated with plasma CRP levels, in particular genes related to the metabolic syndrome (13, 16) such as the leptin receptor gene (*LEPR*) (16, 17). *LEPR* represents an interesting additional candidate gene for a Mendelian randomization study between CRP and adiposity for the following reasons: 1) leptin has been shown to induce the expression of CRP via activation of the leptin receptor in vascular endothelial cells (18), 2) CRP could induce leptin resistance by direct interaction with leptin (19), 3) *LEPR* variants are associated with obesity and fat mass in human (20–22), and 4) leptin signaling, via its receptor, regulates adipose-tissue mass through hypothalamic effects on satiety and energy expenditure. Also, Romero-Corral *et al.* (23) found that the positive association between CRP and cardiovascular disease disappeared after adjustment for leptin levels. Thus, the complex relationships between leptin and CRP require further clarification (23).

Subjects and Methods

Study population

The study was approved by the ethical committee of the University of Lausanne. Recruitment began in June 2003 and ended in May 2006. A simple nonstratified random sample of 35% of the population aged 35–75 yr was drawn, as previously described (24). Among those eligible, the participation rate was 41%. Of the 6188 participants, 826 were excluded because of missing data for BMI (n = 3), CRP (n = 10), genetic information (n = 765), and other covariates (n = 48), leaving 5362 subjects for the present analyses.

Assessment process and data collection

Participants attended the outpatient clinic of the University Hospital of Lausanne in the morning after an overnight fast. Smoking was defined as present if a participant reported to be a current smoker at the time of examination, and alcohol consumption was defined as present for participants reporting to drink alcohol at least once a day. Physical activity was defined as present whenever participants reported to have a physical activity of at least 20 min twice a week or more. Hypertension was defined as having systolic/diastolic blood pressure of 140/90 mm Hg or greater or being on antihypertensive treatment. Diabetes was defined as having fasting glucose of 7.0 mmol/liter or greater or being on antidiabetic medication. We used a dummy variable coded as 0 and 1 for the presence or absence, respectively, of lipid-lowering drugs. The contraceptive pill was coded as 0 or 1 whenever premenopausal women reported not to take (0) or to take (1) the pill and was coded as 0 for postmenopausal women. Postmenopausal hormone replacement therapy was coded as 1 for postmenopausal women who reported having ever taken such therapy and 0 otherwise.

Body weight was measured in kilograms to the nearest 100 g using a Seca scale, which was calibrated regularly. Height was measured to the nearest 5 mm using a Seca height gauge. BMI was defined as weight divided by height in meters squared. Waist circumference was measured with a nonstretchable tape over the unclothed abdomen at the narrowest point between the lowest rib and the iliac crest. Two measures were made and the mean (expressed in centimeters) used for analyses.

Fat and fat-free mass (in percent of the total body weight) were assessed by electrical bioimpedance after a 5-min rest using the Bodystat 1500 body mass analyzer (Bodystat Ltd., Isle of Man, UK). Subjects had to fast for at least 5 h, not engage in strenuous physical activity the previous 12 h, and abstain from consuming caffeine- or alcohol-containing beverages 24 h before the measurement. All metallic adornments were removed, and measurement was performed after a 10-min rest in the lying position. The electrodes were positioned in the right side of the body according to the manufacturer's recommendations. Care was taken to ensure that the subject did not touch any metallic component of the bed and that the inner part of the tights did not touch each other. Fat mass (in kilograms) was calculated from the percentage of fat mass multiplied by weight. Lean mass (in kilograms) was calculated as weight minus fat mass.

A venous blood sample was collected from each participant under fasting conditions. The outpatient clinic of the University Hospital of Lausanne Clinical Laboratory, which is ISO 9001 certified and regularly checked by the Swiss Centre for Quality Control conducted all measurements in a Modular P apparatus (Roche Diagnostics, Basel, Switzerland). High-sensitivity CRP was measured by immunoassay and latex HS (maximum inter- and intrabatch coefficients of variation: 4.6–1.3%) and leptin by ELISA (12.8–5.8%) (American Laboratory Products Co., Windham, NH).

Genotyping and quality controls

Nuclear DNA was extracted from whole blood for whole-genome scan analysis. Genotyping was performed on 6015 participants' samples, using the Affymetrix 500K single-nucleotide polymorphism (SNP) chip, as recommended by the manufacturer (Affymetrix, Santa Clara, CA). Individuals with less than 95% genotyping efficiency overall (or < 90% efficiency on either array, n = 399) and individuals with possible gender inconsistencies (n = 5) were removed. Duplicate samples and first- and second-degree relatives were identified by estimating genomic identity-by-descent coefficients; the younger individual from each such pair was removed (n = 200). Monomorphic SNPs, SNPs with less than 70% genotyping efficiency, SNPs with minor allele frequency less than 1%, and SNPs not in Hardy-Weinberg proportions were excluded from analyses. Twenty-four and 34 SNPs, located within and around the *CRP* and *LEPR* genes, had a minor allele frequency above 10% and were considered for the

analysis (supplemental Tables S1 and S2, published as supplemental data on The Endocrine Society's Journals Online web site at http://jcem.endojournals.org).

Statistical analyses

Statistical analyses were performed using Stata 10.0 (Stata Corp, College Station, TX). Quantitative data were expressed as mean ± SD; qualitative data were expressed as number of subjects (percentage). We used *t* tests and χ^2 tests to compare covariates by sex and analyze the association of SNPs with covariates. CRP levels were log transformed (base 2) to approach the normal distribution. We chose as instrumental variable the SNP with the best results from the linear regression between SNPs and plasma CRP level, assuming an additive model.

To explore the potential causal effect of CRP on adiposity, we used the method of instrumental variables as follows. In a first stage, we regressed CRP on a so-called instrument (see below for the different choices of this instrument in our context). In a second stage, we regressed the response of interest (*e.g.* BMI) on the fitted values from the first stage regression, referred to as explained CRP in what follows. The regression coefficient associated with explained CRP in this second stage can be interpreted as a causal effect of CRP on the response, provided that the instrument is correlated with CRP and that the instrument has no other effect on the response than the effect through CRP. The instrument is said to be weak if the correlation involved in the first assumption above is low, which may result in very large confidence intervals for the regression coefficient. One usually recognizes this issue as a problem if the F test calculated in the first stage regression is less than 10 (25). The second assumption above cannot be verified from the data and should be examined in the context of the particular application. In our case, we have chosen our instruments to be functions of genes that are specifically associated with CRP, such that they are unlikely to have some indirect effect on adiposity. Similar instruments have been used by Timpson *et al.* (26). The method of instrumental variables is also referred to as two-stage least squares (2SLS) or Mendelian randomization.

For the *CRP* gene, the best SNP was *rs7553007*, and for the *LEPR* gene, the best SNP was *rs1805096*. *rs7553007* is located outside the *CRP* gene and *rs1805096* is a synonymous SNP located in exon 20 of the *LEPR* gene. In Haplotype Mapping Project (www.hapmap.org) CEU [Centre d' étude du polymorphisme humain (CEPH), Utah residents with ancestry from northern and western Europe], *rs7553007* is in perfect linkage disequilibrium (*i.e.* perfectly correlated, $D' = 1.0$) with SNP located within the *CRP* gene (*rs1205*) and with *rs2794520* (supplemental figure), which was one of the top SNPs associated with CRP in the Framingham Heart Study genomewide association study (19).

For each association of interest, we conducted both ordinary least square (OLS) regression and 2SLS regression, using the *ivregress* function in Stata 10.0. We compared OLS and 2SLS estimates using the Durbin-Hausman test. We used three different instruments: one SNP from the *CRP* gene region (*rs7553007*), one from the *LEPR* gene region (*rs1805096*), and a combination of the two best SNPs in each gene. *rs7553007* and *rs1805096* were in linkage equilibrium ($D' = 0.03$, $R^2 = 0$). Because we considered these SNPs to be independent, we did not conduct haplotype analyses but summed up their scores using an additive coding (0, 1, 2) for the number of alleles associated with higher CRP levels. This resulted in an ordinal variable with five categories coded from 0 to 4, and a linear effect for this variable was assumed. We conducted unadjusted analyses as well as analyses adjusted for age, sex, BMI, regular alcohol consumption, smoking, physical activity, and postmenopausal hormone replacement therapy. We also conducted additional analyses including leptin as an additional covariate, but this did not change our results (not shown). $P < 0.05$ was considered as statistically significant.

Results

The characteristics of the participants (2526 men and 2836 women) are summarized in Table 1. Overall, men were younger; were more frequently smokers and regular alcohol drinkers; and had higher BMI, lean mass, and waist circumference than women. Women had higher fat mass, CRP, and leptin concentrations. The Spearman rank correlation between circulating CRP and leptin levels was 0.25 in men and 0.40 in women ($P < 0.0001$).

TABLE 1. Participants' characteristics, overall and by gender

	Men (n = 2526)	Women (n = 2836)	*P* value	Total (n = 5362)
Age (yr)	52.9 ± 10.7	53.9 ± 10.7	0.0007	53.4 ± 10.7
BMI (kg/m^2)	26.6 ± 4.0	25.2 ± 4.9	<0.0001	25.9 ± 4.6
Fat mass (kg)	19.9 ± 0.2	23.6 ± 0.2	<0.0001	21.9 ± 8.9
Lean mass (kg)	61.6 ± 8.2	42.9 ± 6.4	<0.0001	51.7 ± 11.8
Waist (cm)	95.9 ± 11.1	83.6 ± 12.5	<0.0001	89.4 ± 13.4
Log2 CRP	0.36 ± 1.53	0.46 ± 1.63	0.0162	0.41 ± 1.59
Log2 leptin[a]	2.72 ± 1.07	3.73 ± 1.09	<0.0001	3.27 ± 1.19
Regular alcohol drinkers (%)[b]	928 (37.7)	443 (15.6)	<0.001	1374 (25.6)
Current smokers (%)	724 (28.7)	690 (24.3)	<0.001	1417 (26.4)
Regular physical activity (%)[c]	1350 (53.4)	1637 (57.7)	0.001	2990 (55.7)
Hormone replacement therapy (%)		1019 (35.9)		

Results are presented as mean ± SD or as number of subjects and percentage. Comparisons were performed using χ^2 or Student's *t* test.

[a] For men, n = 2223; for women, n = 2665.

[b] At least once a day.

[c] At least twice a week.

TABLE 2. Association between the SNPs chosen as instruments and plasma CRP concentration

	Gene	SNP	*P* value[a]	F[a]	R^{2a}
Men	CRP	*rs7553007*	<0.001	31.70	0.0124
	LEPR	*rs1805096*	0.376	0.78	0.0003
	Combined marker	*rs7553007* + *rs1805096*	<0.001	19.54	0.0077
Women	CRP	*rs7553007*	<0.001	25.32	0.0089
	LEPR	*rs1805096*	<0.001	35.54	0.0124
	Combined marker	*rs7553007*+ *rs1805096*	<0.001	65.04	0.0224

[a] *P* value, F, and R^2 are from the first-stage regression in the 2SLS regression analyses.

The statistics from the first-stage regressions between the three variables used as instruments, and log2 CRP, are summarized in Table 2. Except for *rs1805096* in men, the instruments presented sufficient F at first stage regression to be considered as suitable instruments.

Table 3 shows the associations between log2 CRP levels explained by *rs7553007*, the SNP tagging the *CRP* gene, and selected measures of adiposity and lean mass, used as dependent variables. OLS results were all significant in men and women. For the instrumental variable approach (*i.e.* 2SLS), BMI and fat mass were positively associated with log2 CRP levels in women but not men. No such association was observed for lean mass. In women, CRP explained by *rs7553007* was positively, but not significantly, associated with waist circumference. In men, there was evidence that the OLS and 2SLS approaches gave conflicting results and no suggestion of a causal association of CRP with obesity markers. Results were very similar for unadjusted and adjusted analyses. Because *rs7553007* was not associated with diabetes, hypertension status, or use of lipid-lowering drugs, we did not adjust for these variables in the models.

Similarly to what was observed for the *CRP* gene, none of the obesity markers was significantly associated with CRP explained by the *LEPR* instrument in men (Table 4). The large confidence intervals result from the weakness of the *LEPR* instrument, which limits the usefulness of this approach in men. For women, the instrumental variable estimates were lower than those obtained with OLS but still positive and nearly significant for BMI, fat mass, and waist circumference but not for lean mass. In men or in women, *rs1805096* was not associated with diabetes, hypertension status, or use of lipid-lowering drugs. It was weakly associated with circulating leptin ($P = 0.03$) and explained 0.1% of leptin variance, with similar results in men and women. Results were essentially the same when adjusting for leptin in our regression models (data not shown).

For the combined *CRP-LEPR* instrument, no evidence for a causal association was observed in men (Ta-

TABLE 3. Association between CRP and measures of adiposity (with *rs7553007* from the *CRP* gene as instrument)

			OLS		2SLS		
	Dependent variable	Adjusted[a]	β (95% CI)	*P* value	β (95% CI)	*P* value	*P* value[b]
Men	BMI (kg/m^2)	No	0.92 (0.82; 1.02)	<0.001	0.12 (−0.79; 1.02)	0.80	0.08
		Yes	0.86 (0.77; 0.96)	<0.001	0.17 (−0.68; 1.02)	0.70	0.11
	Fat mass (kg)	No	1.78 (1.60; 1.96)	<0.001	−0.65 (−2.47; 1.18)	0.49	0.01
		Yes	1.50 (1.32; 1.68)	<0.001	−0.24 (−1.83; 1.35)	0.76	0.03
	Lean mass (kg)	No	0.56 (0.36; 0.77)	<0.001	1.19 (−0.70; 3.08)	0.22	0.51
		Yes	0.90 (0.69; 1.11)	<0.001	0.85 (−0.92; 2.62)	0.35	0.96
	Waist (cm)	No	2.77 (2.51; 3.04)	<0.001	0.19 (−2.34; 2.72)	0.88	0.04
		Yes	2.41 (2.15; 2.68)	<0.001	0.57 (−1.72; 2.86)	0.63	0.11
Women	BMI (kg/m^2)	No	1.44 (1.34; 1.54)	<0.001	1.22 (0.18; 2.25)	0.02	0.67
		Yes	1.35 (1.25; 1.44)	<0.001	1.29 (0.32; 2.27)	0.01	0.91
	Fat mass (kg)	No	2.78 (2.59; 2.97)	<0.001	2.67 (0.65; 4.68)	0.01	0.91
		Yes	2.52 (2.33; 2.70)	<0.001	2.75 (0.87; 4.63)	0.004	0.81
	Lean mass (kg)	No	0.57 (0.42; 0.71)	<0.001	−0.43 (−1.99; 1.12)	0.59	0.21
		Yes	0.72 (0.59; 0.86)	<0.001	−0.31 (−1.77; 1.14)	0.67	0.16
	Waist (cm)	No	3.61 (3.37; 3.86)	<0.001	2.01 (−0.69; 4.72)	0.14	0.25
		Yes	3.27 (3.03; 3.52)	<0.001	2.11 (−0.40; 4.62)	0.10	0.36

Results are presented as regression coefficient (95% confidence interval) and the *P* value issued from the significance test of the estimate. β, Regression coefficient.

[a] Adjustments are: for both sexes: sex, age, physical activity, regular alcohol consumption, and current smoking, and for women only: hormone replacement therapy.

[b] Value of the test of equality of linear regression and instrumental variables estimates.

TABLE 4. Association between CRP and measures of adiposity (with *rs1805096* from the *LEPR* gene as instrument)

			OLS		2SLS		
	Dependent variable	**Adjusted[a]**	**β (95% CI)**	***P* value**	**β (95% CI)**	***P* value**	***P* value[b]**
Men	BMI (kg/m^2)	No	0.92 (0.82; 1.02)	<0.001	−0.41 (−6.56; 5.75)	0.90	0.67
		Yes	0.86 (0.77; 0.96)	<0.001	0.16 (−3.60; 3.92)	0.93	0.71
	Fat mass (kg)	No	1.78 (1.60; 1.96)	<0.001	3.66 (−7.36; 14.7)	0.51	0.74
		Yes	1.50 (1.32; 1.68)	<0.001	3.57 (−3.65; 10.80)	0.33	0.57
	Lean mass (kg)	No	0.56 (0.36; 0.77)	<0.001	−7.77 (−29.7; 14.2)	0.49	0.46
		Yes	0.90 (0.69; 1.11)	<0.001	−5.17 (−16.9; 6.61)	0.39	0.31
	Waist (cm)	No	2.77 (2.51; 3.04)	<0.001	−1.06 (−18.2; 16.1)	0.90	0.66
		Yes	2.41 (2.15; 2.68)	<0.001	0.70 (−9.39; 10.8)	0.89	0.74
Women	BMI (kg/m^2)	No	1.44 (1.34; 1.54)	<0.001	0.78 (−0.12; 1.67)	0.09	0.15
		Yes	1.35 (1.25; 1.44)	<0.001	0.70 (−0.17; 1.57)	0.11	0.14
	Fat mass (kg)	No	2.78 (2.59; 2.97)	<0.001	1.57 (−0.17; 3.32)	0.08	0.17
		Yes	2.52 (2.33; 2.70)	<0.001	1.35 (−0.32; 3.02)	0.11	0.17
	Lean mass (kg)	No	0.57 (0.42; 0.71)	<0.001	−0.08 (−1.37; 1.21)	0.91	0.33
		Yes	0.72 (0.59; 0.86)	<0.001	0.03 (−1.20; 1.27)	0.96	0.27
	Waist (cm)	No	3.61 (3.37; 3.86)	<0.001	2.15 (−0.13; 4.43)	0.06	0.21
		Yes	3.27 (3.03; 3.52)	<0.001	1.96 (−0.22; 4.14)	0.08	0.23

Results are presented as regression coefficient (95% confidence interval) and the *P* value issued from the significance test of the estimate. β, Regression coefficient.

[a] Adjustments are: for both sexes: sex, age, physical activity, regular alcohol consumption, and current smoking; for women only: hormone replacement therapy.

[b] Value of the test of equality of linear regression and instrumental variable estimates.

ble 5). In women, *CRP* levels explained by this instrument were significantly and positively associated with BMI, fast mass, and waist circumference but not lean mass. In women, none of the instrumental variable estimates significantly differed from OLS estimates, which suggests a causal association of CRP with measures of adiposity. The effect sizes and scales of these associations are illustrated in Fig. 1. The range of CRP levels explained by the combined instrument was much wider in women, in whom clear positive linear trends were observed between explained log2 CRP levels and BMI or fat mass but not lean mass. No association between explained log2 CRP levels and measures of adiposity was found in men.

TABLE 5. Association between CRP and measures of adiposity (with combined marker, *rs7553007* + *rs1805096*, as instrument)

			OLS		2SLS		
		Adjusted[a]	**β (95% CI)**	***P* value**	**β (95% CI)**	***P* value**	***P* value[b]**
Men	BMI (kg/m^2)	No	0.92 (0.82; 1.02)	<0.001	0.04 (−1.12; 1.20)	0.95	0.13
		Yes	0.86 (0.77; 0.96)	<0.001	0.14 (−0.90; 1.18)	0.79	0.17
	Fat mass (kg)	No	1.78 (1.60; 1.96)	<0.001	−0.03 (−1.81; 1.12)	0.98	0.11
		Yes	1.50 (1.32; 1.68)	<0.001	0.48 (−1.36; 2.33)	0.61	0.28
	Lean mass (kg)	No	0.56 (0.36; 0.77)	<0.001	−0.11 (−2.51; 2.30)	0.93	0.58
		Yes	0.90 (0.69; 1.11)	<0.001	−0.37 (−2.58; 1.84)	0.74	0.26
	Waist (cm)	No	2.77 (2.51; 3.04)	<0.001	0.01 (−3.23; 3.26)	0.99	0.09
		Yes	2.41 (2.15; 2.68)	<0.001	0.59 (−2.19; 3.38)	0.67	0.20
Women	BMI (kg/m^2)	No	1.44 (1.34; 1.54)	<0.001	0.98 (0.32; 1.63)	0.004	0.16
		Yes	1.35 (1.25; 1.44)	<0.001	0.97 (0.34; 1.60)	0.002	0.23
	Fat mass (kg)	No	2.78 (2.59; 2.97)	<0.001	2.07 (0.79; 3.34)	0.001	0.27
		Yes	2.52 (2.33; 2.70)	<0.001	1.99 (0.79; 3.19)	0.001	0.39
	Lean mass (kg)	No	0.57 (0.42; 0.71)	<0.001	−0.24 (−1.20; 0.73)	0.63	0.10
		Yes	0.72 (0.59; 0.86)	<0.001	−0.12 (−1.04; 0.79)	0.79	0.07
	Waist (cm)	No	3.61 (3.37; 3.86)	<0.001	2.09 (0.39; 3.78)	0.02	0.08
		Yes	3.27 (3.03; 3.52)	<0.001	2.03 (0.42; 3.63)	0.01	0.12

Results are presented as regression coefficient (95% confidence interval) and the *p* value issued from the significance test of the estimate. β, Regression coefficient.

[a] Adjustments are: for both sexes: sex, age, physical activity, regular alcohol consumption, and current smoking; for women only: hormone replacement therapy.

[b] Value of the test of equality of linear regression and instrumental variables estimates.

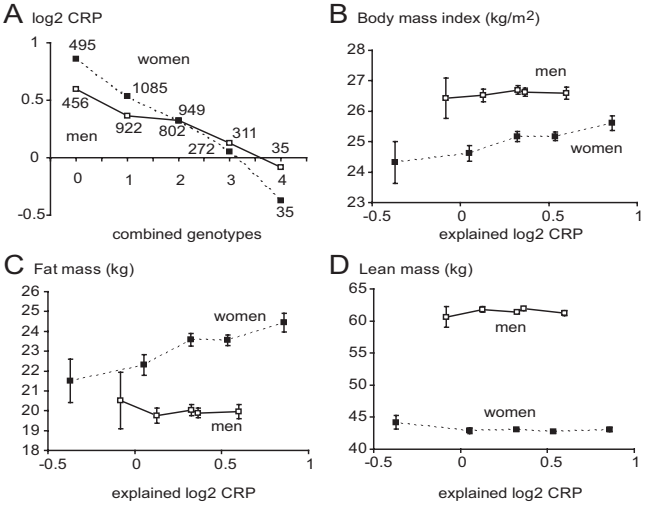

FIG. 1. Illustration of the relations between log2 CRP, measures of adiposity, and combined *CRP-LEPR* instrument, by sex. *Squares* are means and *bars* are SES. The combined instrument is the sum of the SNP around the *CRP* gene (*rs7553007*) and the SNP from the *LEPR* gene (*rs1805096*), each coded using an additive mode of action of the allele associated with higher CRP level. The combined instrument has five categories (ranging from 0 to 4), which represent the number of alleles associated with higher CRP level. A, Mean (SE) log2 CRP, depending on the combined *CRP-LEPR* instrument, by sex. *Numbers above and below squares* represent sample sizes. B–D, Values of mean (SE) BMI, fat mass, and lean mass, respectively, depending on the mean CRP for each category of the combined instrument, by sex.

Discussion

CRP explained by a single SNP (*rs7553007*) tagging the *CRP* gene was positively associated with BMI, waist circumference, and fat mass in women, whereas no such evidence was found in men. These results suggest a causal association of CRP with BMI in women. CRP explained by *rs7553007* probably represents lifelong CRP levels free of confounding or reverse causation. The selective association with fat mass, but not with lean mass, reinforces our confidence that our results are not spurious. Recently Timpson *et al.* (26) found conflicting results between conventional least squares regression analysis and instrumental variable approach for the association between BMI and CRP in a large sample of postmenopausal British women. Their results suggested that there was no causal association between CRP and BMI in this group. Important differences between our study and theirs may explain this discrepancy: 1) age range of women was 35–75 *vs.* 60–79 yr, respectively, 2) different *CRP* genetic markers were used, and 3) average BMI and CRP were lower in our study.

Lange *et al.* (27) have shown that SNPs located within the *CRP* gene are associated with plasma CRP levels and cardiovascular mortality, and this latter association persisted even after adjustment for plasma CRP levels. In the latter study (27), *CRP* SNPs accounted for less than 2% of the CRP variability. Lange *et al.* (27) postulated that plasma CRP measured at a single point in time may not reflect lifelong CRP levels, whereas *CRP* SNPs associated with CRP levels may better capture an individual's cumulative inflammatory burden. Our SNP (*rs7553007*) is in linkage disequilibrium ($D' = 0.92$, based on HapMap CEU release 22 data) with the *CRP* tagging SNP (*rs1417938*) found to be associated with cardiovascular mortality and stroke in Caucasians in this latter study (27).

It has been suggested that CRP could induce leptin resistance via direct physical interaction (19). We found a positive correlation between circulating leptin levels and CRP, which was much stronger in women ($r = 0.40$) than men ($r = 0.25$). Because of the tight interrelationships between leptin and CRP (19) and because genetic variants in the *LEPR* gene have been associated with CRP levels (16, 17), we also analyzed the associations of SNPs located within the *LEPR* gene with CRP levels using Mendelian randomization. The leptin receptor is the effector of leptin action and its ubiquitous expression in the body reflects the pleiotropic actions of leptin. The leptin receptor exists in several alternative spliced forms. Therefore, genetically determined variability in the leptin receptor represents an indirect way of capturing varying degrees of leptin actions, with a focus on the biologically active fraction of leptin. In women, CRP explained by the *rs1805096 LEPR* variant was also positively, although not significantly, associated with BMI, fat mass BMI, and waist circumference (see Table 4). *rs1805096* has already been previously associated with CRP levels and other markers of inflammation in Caucasians (17). By contrast, no such tendency was observed with lean mass. These results were in line with those obtained for the *CRP* gene.

In women, the association of CRP explained by *LEPR* variants with adiposity changed only moderately after adjusting for leptin, which suggests that this association is independent of cross-sectional leptin levels. Similarly to what has been stated for *CRP* gene variants and lifelong CRP levels, *LEPR* gene variants may better reflect the lifelong effects of leptin than leptin levels measured at a single point in time. For this gene, results in men were difficult to interpret because the best variant explained only an extremely small proportion of variance (F test <10 at first stage regression). In this case, Mendelian randomization is of little use because of the weakness of the genetic instrument.

Our results suggest that leptin may be associated with adiposity, in part, via CRP. Such a direct action of leptin on CRP has already been suggested by previous findings (17–19). Leptin induces acute phase protein genes in hepatic cell lines overexpressing the leptin receptor (28). Experimental data in humans show that the administration of leptin slightly, but selectively, increase CRP levels (29).

A Mendelian randomization study including genetic variants in the *CRP* gene found no evidence for CRP as a causal agent for leptin (30). These latter findings are consistent with our results. Clearly, further evidence is needed to clarify the complex relationship between leptin and CRP.

The instrumental variable created by combining the *CRP* and *LEPR* instruments explained a larger proportion of CRP variance in women (2.25%) but not men (0.7%). This analysis confirmed with higher precision the effects observed for the *CRP* instrument and, to a lesser extent, the *LEPR* instrument in women. Results in men were inconclusive, although not significantly different from those obtained in women. Our results confirm the heterogeneity of the genetic determinants of CRP (16) and suggest that both SNPs (*rs7553007* and *rs1805096*) contribute independent information to the relationship between CRP and measures of adiposity.

The putatively causal association between CRP and measures of adiposity was significant only in women. However, the results were not significantly different in men and women (*i.e.* confidence intervals for their respective regression parameters largely overlapped). The results of the Mendelian randomization approach were very imprecise in men, and it is possible that we did not have enough power to detect a weak effect. A potential explanation for the observed sex difference is that women have higher leptin and CRP levels and a higher proportion of fat mass than men. Some authors suggested that leptin could explain the gender difference in CRP levels (31, 32). It is therefore possible that, at the low leptin levels found in men, the causal association between CRP and adiposity cannot be detected via instruments as weak as the one we used. In addition, the association between fat mass and CRP using instrumental variables tended to be stronger in postmenopausal than premenopausal women, who have lower leptin levels than postmenopausal women. Examples of sex differences in the determinants of obesity have been previously reported in experimental mice models (33) and in humans (34). Men and women differ in their body fat distribution, and this is primarily attributable to the action of sex steroids (35). Adult women have higher body fat mass and lower lean mass than men. Fat pattern is usually android in men (*i.e.* fat accumulation in the abdominal region) and gynoid in women (*i.e.* fat accumulation in the gluteal and femoral regions).

Our study suffers from several limitations. First, genetic markers were part of a 500K Affymetrix chip, which was not specifically designed to cover the selected genes. Hence, it is likely that we did not measure the best markers for these genes. We also do not know which causal variant plays a role. However, it is remarkable that, despite this limitation, we found such results. Second, the SNPs explained only about 1% of the CRP variance, which would make them weak instruments for Mendelian randomization. However, the proportion of CRP variance genetically explained in our study is in line with findings in recent large-scale studies (16, 36), and *rs7553007* is in perfect linkage disequilibrium with *rs1205* in HapMap ($r^2 = 1.0$), a SNP that was recently found to capture the strongest effects, of eight SNPs including *rs1417938* and *rs1130864*, of the CRP gene on circulating CRP levels (37). An important consequence of this is that estimates from the instrumental variable approach have extremely wide confidence intervals. Third, the validity of the Mendelian randomization approach should be evaluated in the context of the study. In our context, problems could be developmental canalization (*i.e.* a genetic variant could induce compensatory phenomena leading say to CRP resistance) or heterogeneous effects in the presence of linkage disequilibrium (38, 39). This could be of particular concern for the *LEPR* gene. Our results should therefore be taken with caution. Fourth, our results may not apply to non-Caucasians because the association of genetic variation in the *CRP* gene with plasma CRP levels significantly differs across ethnic groups (40).

Conclusion

To our knowledge, this is the first study in which the association between CRP and BMI was partly explained by variations in the *CRP* gene. Our results suggest that CRP is causally related to BMI in women and that this is mainly due to fat mass. Results in men were not significant. Results on the combined CRP-LEPR instrument suggest that leptin may play a role in the causal association between CRP and BMI. The fact that these relationships were observed only in women may be explained by the higher leptin levels in women because CRP could be involved in leptin resistance.

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