CLINICAL STUDY

Epidemiological evidence against a role for C-reactive protein causing leptin resistance

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Abstract

Objective: It has been suggested that elevated levels of C-reactive protein (CRP) might interfere with leptin signalling and contribute to leptin resistance. Our aim was to assess whether plasma levels of CRP influence leptin resistance in humans, and our hypothesis was that CRP levels would modify the cross-sectional relationships between leptin and measures of adiposity.

Design and methods: We assessed four measures of adiposity: BMI, waist circumference, fat mass and body fat (%) in 2113 British Regional Heart Study (BRHS) men (mean (s.d.) age 69 (5) years), with replication in 760 (age 69 (6) years) European Male Ageing Study (EMAS) subjects.

Results: In BRHS subjects, leptin correlated with CRP (Spearman's $r=0.22$, $P<0.0001$). Leptin and CRP correlated with all four measures of adiposity (r value range: 0.22–0.57, all $P<0.0001$). Age-adjusted mean levels for adiposity measures increased in relation to leptin levels, but CRP level did not consistently influence the β -coefficients of the regression lines in a CRP-stratified analysis. In BRHS subjects, the BMI vs leptin relationship demonstrated a weak statistical interaction with CRP ($P=0.04$). We observed no similar interaction in EMAS subjects and no significant interactions with other measures of adiposity in BRHS or EMAS cohorts.

Conclusion: We have shown that plasma CRP has little influence on the relationship between measures of adiposity and serum leptin levels in these middle-aged and elderly male European cohorts. This study provides epidemiological evidence against CRP having a significant role in causing leptin resistance.

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Introduction

In health, leptin produced by adipose tissue has a role in suppressing appetite and preventing weight gain (1). Leptin production is strongly related to short-term

calorie intake (2) and fat mass (3). Overweight and obese individuals tend to have higher leptin levels compared with lean people, and they appear to be resistant to the central hypothalamic effects of elevated leptin levels. This reduced sensitivity to endogenous

leptin has been described as 'leptin resistance' (4). Although this state is usually associated with overweight or obesity, it has also been described in normal-weight overfed animals (2).

The clinically important molecular mechanisms causing leptin resistance in humans are uncertain. Leptin exerts its effect by binding and activating leptin receptors in the hypothalamus and other organs. Leptin resistance has been shown to be influenced by altered transport of leptin across the blood–brain barrier (5, 6, 7), the presence of different isoforms of the leptin receptor and by altered expression of molecules that impair leptin signalling (e.g. p-STAT and SOCS-3) (6, 8).

Based on *in vitro* data, Chen *et al.* (9) suggested that C-reactive protein (CRP) is one of the major serum proteins interacting with leptin and that in cell culture human CRP blocks leptin signalling. They showed that leptin stimulates production of CRP by hepatocytes, which could lead to clinically important leptin resistance if CRP was to block leptin signalling. Chen also presented data showing that in leptin-deficient mice, infusion of human CRP blocked weight reduction associated with leptin administration, and the actions of human leptin were blunted in mice expressing a transgene encoding human CRP. Subsequent work has suggested that the reported physical binding of leptin to CRP is likely to have been an artefact (10) and other work has failed to demonstrate a significant effect of CRP on leptin action *ex vivo*, or *in vivo*, or of leptin on circulating CRP levels in humans *in vivo* (10, 11, 12). However, if CRP was to block leptin signalling, this could have far-reaching implications.

It is firmly established that obesity leads to increased circulating inflammatory cytokines that act on the liver increasing hepatic CRP production (13). If CRP was to have a role in leptin resistance, then this would provide important insights into the pathogenesis of obesity and type 2 diabetes and it would help to explain why obese individuals find weight loss difficult to achieve. It would also suggest novel therapeutic targets to reduce obesity and type 2 diabetes.

While there is some *in vitro* data refuting the hypothesis that CRP has a role in leptin resistance, the absence of epidemiological data in humans has been highlighted (14). Our aim was to assess whether plasma CRP influences the relationship between leptin and measures of adiposity in two large population-based cohorts. Our hypothesis was that if CRP causes leptin resistance, then it would influence the relationship between adiposity and leptin.

Materials and methods

We performed our initial analysis in British Regional Heart Study (BRHS) participants and repeated the analysis in European Male Ageing Study (EMAS) subjects applying similar inclusion criteria. In both studies, BMI was calculated as body weight (kg)/the

square of height (m^2). Local Ethics Committees, functioning according to the 3rd edition of the Guidelines on the Practice of Ethics Committees in Medical Research issued by the Royal College of Physicians of London, approved all studies. All men provided informed written consent to the investigations carried out after full explanation of the purpose and nature of all procedures used and in accordance with the Declaration of Helsinki.

British Regional Heart Study

The BRHS is a prospective study of cardiovascular disease involving 7735 men, aged 40–59 years, selected from the age–sex registers of one general practice from each of 24 British towns between 1978 and 1980 (15). The data presented here are from the 20th year review (1998–2000) to which all surviving men were invited. Of the 4252 men (77% of survivors) who attended, we excluded those with diabetes (physician diagnosis or fasting glucose ≥ 7 mmol/l); a physician diagnosis of coronary heart disease or cancer; those with CRP level > 10 mg/l (to reduce confounding by infection and chronic inflammatory disease) and those treated with aspirin, leaving 2113 men available for analysis.

Details of fasting blood sampling, anthropometry and estimation of fat mass by bioelectrical impedance have been described previously (16, 17, 18). CRP was assayed by ultrasensitive nephelometry (Dade Behring, Milton Keynes, UK), with intra- and inter-assay coefficients of variation (CV) of 4.7 and 8.3% respectively and a detection limit of 0.15 mg/l (17). Plasma leptin was measured by an in-house RIA validated against the commercially available Linco assay (19) with CV of < 7 and $< 10\%$ and a detection limit of 0.5 ng/ml (17).

European Male Ageing Study

The EMAS is a population-based prospective cohort study of 3369 men, aged 40–79 years, recruited from population registers of eight European countries (UK, Sweden, Estonia, Poland, Hungary, Italy, Spain and Belgium) (20). After applying similar selection criteria as used in BRHS subjects, 760 men aged < 60 years at baseline examination 2003–2005 were available for this analysis. In the EMAS cohort, diabetes was defined as self-reported diabetes, a fasting plasma glucose ≥ 7.0 mmol/l or treatment for diabetes.

Anthropometry and fasting venous blood samples were obtained in all subjects (20). Fat mass was assessed from skin-fold thicknesses measured at four anatomical sites (21). CRP was assayed by immunoassay (Immulite 2000 high-sensitivity assay, DRG Instruments GmbH, Marburg, Germany) with intra- and inter-assay CV of 2.8 and 3.1% respectively and a detection limit of 0.1 mg/l. Leptin was assayed by a sandwich ELISA assay (DRG Leptin (sandwich) assay, DRG Instruments GmbH) with CV of 6.9 and 11.6% and a detection limit of 1.0 ng/ml.

Figure 1 Anticipated relationships between adiposity measures and leptin levels stratified by CRP tertile if (a) CRP does not cause leptin resistance and (b) if CRP does cause leptin resistance.

Statistical analysis

We assessed four measures of adiposity: BMI, waist circumference, fat mass and percentage body fat. The distributions of leptin and CRP were highly skewed and we therefore considered quintiles of leptin and tertiles of CRP or $\log_e$ -transformed data when appropriate. We used Spearman's rank correlation coefficients to assess the relationship between measures of adiposity and leptin or CRP because transformation of the data before Pearson's correlation may have influenced the relationships differently within BRHS and EMAS cohorts, and our aim was to assess whether these relationships were similar within each cohort.

We assumed that if CRP influenced leptin resistance, then the gradient of the relationship between leptin and measures of adiposity would be modified by CRP. Figure 1 describes two hypothetical scenarios in which CRP either i) does not or ii) does have a role in leptin resistance. In the former situation, we suggest that a plot of leptin vs adiposity, stratified by CRP tertile, would show three parallel lines because CRP is positively related to adiposity at all leptin concentrations, and CRP level would not influence the strength of these relationships (Fig. 1a). However, if increasing CRP concentrations had increasing effects on leptin resistance, then we suggest that the gradient of the leptin–adiposity plot would be modified by the CRP level as shown in Fig. 1b. The three lines on the figure would not cross because the relationship between CRP and adiposity would remain positive at all leptin concentrations.

Analysis of covariance was used to obtain age-adjusted means of the adiposity measures by quintiles of leptin stratified by tertiles of the CRP distribution. We obtained age-adjusted β -coefficients for the linear regression equation between measures of adiposity and leptin; the potential modifying effect of CRP was assessed from the change in β -coefficient for these relationships across low, medium and high CRP strata. We assessed the influence of CRP in the relationships between leptin and adiposity by assessing the statistical significance of the interaction term ($\log_e \text{leptin} \times \log_e \text{CRP}$) in the following regression models:

adiposity variable = age + $\log_e \text{CRP}$ + $\log_e \text{leptin}$ + ($\log_e \text{leptin} \times \log_e \text{CRP}$).

We estimated that each analysis required 597 subjects to detect a small statistical effect (R^2 (f^2) = 0.02) of the interaction term ($\log_e \text{CRP} \times \log_e \text{leptin}$) in the models listed earlier that included four predictor variables (assuming, $\alpha=0.05$; power=0.8). A two-sided $P<0.05$ was considered statistically significant. We performed analyses using SAS (version 8.2; SAS Institute, Cary, NC, USA).

Results

Clinical characteristics, leptin and CRP levels were similar in BRHS and EMAS subjects (Table 1). Leptin correlated with CRP in both studies (BRHS, $r_s=0.22$; EMAS, $r_s=0.21$; both $P<0.001$). Leptin and CRP were significantly associated with all adiposity measures; leptin being more strongly related than CRP (Table 2).

To illustrate the influence of CRP on the relationships between leptin and adiposity, Fig. 2 shows the age-adjusted mean levels for the adiposity measures (BMI, WC, fat mass and body fat (%)) by quintiles of leptin levels, stratified by tertiles of CRP in the BRHS subjects. Subjects with higher CRP levels tended to have higher levels of adiposity for any given leptin level. Age-adjusted mean levels for adiposity measures increased with increasing levels of leptin in all CRP groups. These patterns of associations were also seen in the EMAS cohort (data not shown).

Table 3 shows the β -coefficients of the regression lines between leptin and each of the adiposity measures in the CRP-stratified analysis in the BRHS and EMAS cohorts separately.

The increase in BMI with increasing leptin appeared to be steeper for subjects who had high CRP levels in the BRHS cohort. In BRHS subjects, the BMI–leptin relationship demonstrated a weak interaction with CRP (P value for the ($\log_e \text{leptin} \times \log_e \text{CRP}$) interaction term = 0.04; Table 3). A non-significant CRP interaction was observed in the relationships between waist and leptin ($P=0.06$) and no CRP interaction observed between leptin and fat

Table 1 Participant characteristics. Data are mean (s.d.) or geometric means (IQR).

Variable	BRHS	EMAS
<i>n</i>	2113	760
Age (years)	68.0 (5.4)	69.4 (5.6)
Weight (kg)	79.0 (11.9)	81.3 (12.3)
Height (cm)	172.6 (6.5)	170.9 (6.7)
BMI (kg/m ²)	26.5 (3.4)	27.8 (3.6)
Waist circumference (cm)	95.9 (9.7)	99.7 (10.0)
Fat mass (kg)	27.5 (9.0)	22.9 (6.5)
Body fat (%)	34 (8)	28 (5)
Leptin (ng/ml)	8.5 (5.4–13.2)	5.6 (2.5–13.9)
C-reactive protein (mg/l)	1.4 (0.7–2.8)	2.9 (1.5–5.8)

BRHS, British Regional Heart Study; EMAS, European Male Ageing Study.

Table 2 Rank correlation of adiposity measures with leptin and CRP.

Variable	BRHS		EMAS	
	Leptin	CRP	Leptin	CRP
Age	0.03*	0.15	0.06*	0.14
BMI	0.57	0.22	0.56	0.17
Waist circumference	0.56	0.26	0.58	0.20
Fat mass	0.52	0.22	0.56	0.17
Body fat (%)	0.44	0.22	0.60	0.16

Data are Spearman's coefficients; all $P < 0.0001$ unless stated; * $P = NS$. BRHS, British Regional Heart Study; EMAS, European Male Ageing Study.

mass ($P = 0.13$) or body fat (%) ($P = 0.87$). However, in the EMAS cohort, there were no statistically significant interactions with CRP for any of the relationships between adiposity measures (BMI, waist circumference, fat mass or body fat (%)) and leptin ($P > 0.22$ for all the ($\log_e$ leptin $\times \log_e$ CRP) interaction terms).

Discussion

Principal findings

To our knowledge, this is the first epidemiological study in adults to assess the potential role of CRP in leptin resistance. In our two large cohorts of middle-aged and elderly men, we confirmed that mean adiposity levels were related to leptin, and we showed that CRP levels had no clinically important influence on these relationships. There was little evidence that CRP exerts a statistically significant interaction on relationships between leptin and several adiposity measures. Overall, we conclude that in these male cohorts, our data support the notion that CRP has no clinically important role in causing leptin resistance.

Data interpretation

In our analysis of BRHS subjects, we showed evidence of a weak statistical interaction of CRP in the leptin–BMI relationship ($P = 0.04$) but not in the other leptin–adiposity relationships studied (Table 3). Figure 2 suggests that the significant statistical interaction of CRP in the leptin–BMI relationship is due to the finding that CRP does not relate to BMI except at higher leptin levels. Indeed, in those with low leptin, CRP does not relate to any adiposity measures. These altered relationships occur only at the lower end of the adiposity distributions and appear to explain the interaction in the overall model presented in Table 3. This interaction is not observed in the overweight or obese range and therefore it is unlikely to be of clinical significance.

However, these observations prompted us to validate our results in the EMAS cohort. When we applied near-identical selection criteria, we showed no evidence of any interaction of CRP in the leptin–adiposity relationships. We believe that the absence of interactions in our

validation cohort strongly suggests that CRP has no clinically important effect on leptin resistance.

Sample size/power

The negative relationships in our data raise the question of statistical power. However, i) we had adequate power in either BRHS or EMAS cohorts to detect a small statistical interaction ($R^2 = 0.02$) in the four-variable models presented in Table 3 and ii) we studied sufficient numbers of subjects to exclude a clinically important influence of CRP in the leptin–adiposity relationships.

Other evidence

The report by Chen *et al.* (9) is controversial. Several groups have tried unsuccessfully to replicate the findings: first, molecular binding of CRP to leptin has not been confirmed (11, 12). Secondly, in experiments using leptin-sensitive cells, CRP had no effect on the proliferation and function (12), and thirdly, in mice, the co-administration of CRP with leptin showed no blunting of appetite suppression or weight loss (11). Lastly, in patients with congenital leptin deficiency, plasma CRP levels did not change on leptin administration (10). The notion that CRP has a role in causing leptin resistance also lacks biological plausibility because of the large range of plasma CRP concentrations (10 000-fold) and the rapid rise that occurs during acute illness (11).

Two recent studies have explored the potential role of CRP in leptin resistance. The first was a cross-sectional study in 51 pre- or peri-pubertal girls, and it claimed that CRP contributed to leptin resistance because CRP accounted for 10% of the variance in leptin levels after adjusting for potential confounders including adiposity (22). However, the authors did not assess whether CRP levels modified the relationships between leptin and measures of adiposity. The second study showed that CRP blunted the *in vitro* production of nitric

Figure 2 Cross-sectional relationships between age-adjusted mean values for adiposity measures and leptin quintiles stratified by CRP tertile in 2113 BRHS men.

Table 3 β -Coefficients from the age-adjusted linear regression equation between adiposity and leptin within CRP strata, and the *P* value for interaction term*.

Cohort	Adiposity measure	CRP tertile (β -coefficient (S.E.M.))			<i>P</i> value*
		Low	Medium	High	
BRHS	BMI	2.51 (0.14)	2.46 (0.15)	2.95 (0.17)	0.04
	Waist	7.09 (0.40)	6.38 (0.41)	8.42 (0.49)	0.06
	Fat mass	6.25 (0.37)	5.78 (0.39)	7.03 (0.49)	0.13
	% body fat	4.86 (0.36)	4.16 (0.37)	4.67 (0.43)	0.87
EMAS	BMI	1.51 (0.14)	1.47 (0.17)	1.78 (0.15)	0.22
	Waist	4.75 (0.41)	4.20 (0.45)	4.90 (0.41)	0.47
	Fat mass	2.90 (0.25)	3.16 (0.30)	3.31 (0.27)	0.26
	% body fat	1.90 (0.20)	2.30 (0.22)	2.14 (0.18)	0.46

**P* value for ($\log_e$ leptin $\times$ $\log_e$ CRP) interaction term in the relationship: adiposity variable = age + $\log_e$ CRP + $\log_e$ leptin + ($\log_e$ leptin $\times$ $\log_e$ CRP). *P* values (trend) <0.0001 for all β -coefficients. BRHS, British Regional Heart Study; EMAS, European Male Ageing Study.

oxide by leptin in human aortic endothelial cells (23). While this study has several strengths, the relevance of these findings to the hypothalamus, and in particular, to human obesity is unclear.

In conclusion, the data from these and other sources provide no strong evidence that CRP has a role in leptin resistance.

Strengths, limitations and design of future studies

We have assessed the influence of CRP on the cross-sectional relations of leptin and four measures of adiposity in two large population-based cohorts. The results of our analyses were similar in our two cohorts, even though there were some differences in the clinical assessments and the laboratory methods used. We have used several methods to assess whether CRP modifies the relationship between leptin and measures of adiposity.

The cross-sectional design and our relatively healthy, mostly overweight, middle-aged and elderly male subjects are study limitations. It would seem appropriate to test this hypothesis in prospective cohorts of younger individuals, including women and in larger number of people with obesity. In experimental animals, leptin resistance is associated with increasing age (24, 25, 26), and therefore, it is appropriate that we studied older individuals.

Two of our methods of assessing the role of CRP in leptin resistance rely on the assumption that the action of leptin on adiposity is blunted by CRP in a concentration-dependent manner. While we believe that this is the most biologically plausible mechanism, other possibilities exist, and this would influence our conclusions.

The design of our studies meant that we did not have access to biopsy specimens to assess levels of signalling molecules downstream of the leptin receptor. However, the only tissues that might be conceivably suitable for large-scale epidemiological studies would be fat biopsy samples.

As this tissue might behave differently to hypothalamic tissue, the value of this data would be uncertain.

The between-study differences in CRP and leptin are likely to be explained by differences in the populations studied and the use of different leptin and CRP assays (27, 28). When compared with BRHS men, EMAS men had a higher mean BMI and waist but lower mean fat mass. The difference in fat mass is likely to be explained by the different methods used (BRHS, bioelectrical impedance; EMAS, skin-fold thicknesses). We believe that these between-study differences have not influenced our conclusions because we performed each analysis within the individual cohort. We were reassured by the degree of agreement between the studies in the rank correlations presented in Table 2 and in the consistent ratio of the β -coefficients of between-studies (Table 3).

Conclusions

We find little evidence for an effect of CRP on the relationships between adiposity and leptin levels in two large cohorts providing further evidence against the notion that CRP plays a role in causing leptin resistance.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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