



PAPER

C-reactive protein is independently associated with total body fat, central fat, and insulin resistance in adult women

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OBJECTIVE: To investigate whether C-reactive protein (CRP) concentrations are influenced by body composition, insulin resistance, and body fat distribution in healthy women.

DESIGN: Cross-sectional study of CRP plasma levels in adult women.

SUBJECTS: A total of 201 apparently healthy normal weight, overweight, and obese women, aged 18–60y.

MEASUREMENTS: CRP plasma levels, several fatness and body fat distribution parameters (by bioimpedance analysis and anthropometry), and insulin resistance (HOMA_{IR}), as calculated by homeostatic model assessment.

RESULTS: CRP was positively correlated with age, body mass index (BMI), waist, fasting glucose and insulin, HOMA_{IR}, fat-free mass (FFM) and fat mass (FM). After multivariate analyses, age, HOMA_{IR}, waist and FM maintained their independent association with CRP.

CONCLUSION: Our study has shown an independent relationship of central fat accumulation and insulin resistance with CRP plasma levels, thus suggesting that mild, chronic inflammation may be a further component of the metabolic syndrome and a mediator of the atherogenic profile of this syndrome.

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Keywords: C-reactive protein; body composition; total body fat; central fat; insulin resistance

Introduction

C-reactive protein (CRP) is an acute-phase protein produced by hepatocytes in response to tissue injury, infection, and inflammation.¹ It is noteworthy that CRP plasma levels even below the conventional upper limit of normal (1 mg/dl) have been associated with a 2–3-fold increase in risk of future myocardial infarction, stroke, and peripheral atherosclerosis among apparently healthy middle-aged men and women.^{2–4}

The relationship between CRP and cardiovascular disease is more than a mere epiphenomenon; in other words, this acute-phase reactant is not just a marker of increased inflammatory activity, but it is also directly involved in the pathogenesis of atherothrombosis, through several mechanisms

(ie binding of plasma lipoproteins, activation of complement, induction of tissue factor).^{5–7}

Recently, CRP concentrations have been shown to be significantly associated with several cardiovascular risk factors, such as age, smoking, hypertension, exercise, plasma lipids, homocyst(e)ine, and body mass index (BMI).⁸ Concerning the relationship between CRP concentration and BMI level, the Third National Health and Nutrition Examination Survey found that the prevalence of elevated CRP levels (ie CRP concentrations ≥ 0.22 mg/dl) is higher both in overweight (BMI 25–29.9 kg/m²) and obese (BMI ≥ 30 kg/m²) patients than in normal weight (BMI < 25 kg/m²) subjects.⁹

It has been suggested that the association between BMI and CRP might be mediated by cytokines, such as interleukin-6 (IL-6) and tumor necrosis factor α (TNF- α), which are both expressed in adipose tissue^{10,11} and referred to as main regulators of CRP production in the liver.^{12,13} Indeed, levels of CRP have been found to be significantly related to IL-6 and TNF- α concentrations, as well as to *insulin resistance score*

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and markers of endothelial dysfunction (ie plasma levels of vonWillebrand factor, tissue plasminogen activator, and cellular fibronectin) in nondiabetic subjects.¹⁴

To further explore the relationship between inflammation and body fatness, we performed a study evaluating concentrations of CRP in healthy normal-weight, overweight, and obese women, aged 18–60y, free of reported cardiovascular disease. We also sought to determine the relationships of CRP with body fat distribution, body composition, as evaluated with bioimpedance analysis, and insulin resistance, as calculated from homeostatic model assessment (HOMA).¹⁵

Subjects

The study included 201 women [(48 with BMI < 25.0 kg/m² (normal weight), 37 with BMI ≥ 25.0 kg/m² and < 30.0 kg/m² (overweight), and 116 with BMI ≥ 30.0 kg/m² (obese)], aged 18–60y. All subjects gave their informed consent to be included in the study, which was performed in accordance with the guidelines proposed in the Declaration of Helsinki. Overweight and obese patients were recruited consecutively at the Outpatient Clinic for the Study of Obesity, Department of Emergency and Transplant, Section of Internal Medicine, Endocrinology and Metabolic Diseases, University of Bari, School of Medicine. Normal weight subjects were represented by healthy volunteers, recruited consecutively among physicians and medical students. The pre-menopausal women involved in the study were spontaneously menstrually active (9–13 menses per year). None of the participants were diabetic, in accordance with the Report of the Expert Committee on the Diagnosis and Classification of Diabetes Mellitus,¹⁶ and only five out of 201 women had impaired glucose tolerance (IGT). To the aim of avoiding the confounding effect of other cardiovascular risk factors, smokers and patients affected by stable hypertension and familial hyperlipidemia were not included in the study. Moreover, none of the subjects had positive clinical history of stroke, transient ischaemic attack, angina pectoris, heart infarction, claudicatio intermittens, congenital heart disease or ECG abnormalities. Biochemical markers of thyroid, liver, and kidney function were within normal range in all subjects, and none of individuals were receiving any kind of drug (including oral contraceptives for pre-menopausal women and hormone replacement therapy for post-menopausal women) when they entered into the study. During the testing period, all subjects were asked to keep their normal mixed diet and not to perform any sporting activity.

Methods

Central fat accumulation was evaluated by the waist circumference, measured as the minimum circumference between the xyphoid process and the umbilicus. Blood pressure was

recorded on at least three different occasions, using a mercury manometer with an appropriate cuff size.

Blood for hormonal and metabolic determinations was drawn between 8 and 9 am after overnight fasting, on day 5–7 of the menstrual cycle in pre-menopausal women. The oral glucose tolerance test (OGTT) was performed by collecting venous blood samples during fasting and every 30 min following oral load with 75 g glucose for 2 h.

Plasma concentrations of insulin were measured by radioimmunoassay, using a commercially available kit (Behring, Scoppitto, Italy). Blood glucose was determined by the glucose-oxidase method (Sclavo, Siena, Italy). Total cholesterol, HDL-cholesterol, and triglycerides were measured using enzymatic assays (Boehringer Mannheim, GmbH Diagnostica Mannheim, Germany). Both intra-assay and the inter-assay Cvs were less than 7.5% in all of the above methods.

Insulin resistance was measured from fasting glucose and insulin concentrations with HOMA, as previously described.¹⁵ CRP protein was measured with use of latex-enhanced immunonephelometric method. Both within- and between-assay quality control procedures were used and the coefficient of variation of the method was 3.2–16.1% through the period of the data collection.

Body composition was estimated fasting by the bioimpedance method using a tetrapolar device (BIA 101/S, RJL Systems, Detroit, USA; Akern s.r.l., Florence, Italy). The fat-free mass (FFM) was calculated by Heitmann's equation,¹⁷ and fat mass (FM) was calculated as the difference between body weight and FFM.

Statistical methods

Statistical analysis was performed using the STATISTICA[®] 6.0 for Windows, StatSoft Inc. (1995) software (Tulsa, OK, USA). Results are presented as mean ± standard error for all parameters. The K–S test and the Lilliefors test for normality were performed to check whether the wide range of BMI of the whole population, including both normal weight and obese subjects, could be a sign of a bimodal distribution; since both the tests were not significant ($P > 0.2$), it is possible to argue that these two different populations could be tested together. Pearson's correlation coefficients were used to quantify the univariate associations among variables. Multiple regression analysis was performed to test the joint effect of age, anthropometric, metabolic, and body composition parameters on CRP levels (dependent variable). Variables not normally distributed (insulin and triglycerides) were logarithmically transformed for simple correlations and multivariate analysis. The difference in CRP plasma concentrations among quartiles in which the whole population was divided according to HOMA_{IR} levels was analysed using one-way analysis of variance (ANOVA). The minimal statistical significance was defined as $P < 0.05$.

Results

General, anthropometric, and metabolic characteristics of the population are shown in Table 1. Table 2 displays the correlation coefficients between CRP plasma levels and all the variables investigated. CRP was positively correlated with age ($P < 0.001$), BMI ($P < 0.0001$), waist circumference ($P < 0.0001$), fasting glucose ($P < 0.0001$), fasting insulin ($P < 0.0001$), HOMA_{IR} ($P < 0.0001$), FFM ($P < 0.0001$), and FM ($P < 0.0001$); whereas, it was associated neither with blood pressure (systolic and diastolic) nor with plasma lipids (total cholesterol, HDL-cholesterol, and triglycerides).

When CRP was considered as a dependent variable in a forward-stepwise multiple regression analysis with age, BMI (or waist circumference), total cholesterol, HDL-cholesterol, triglycerides, fasting glucose, fasting insulin, FFM, and FM as independent variables (fitted model: adjusted r^2 0.237, F -ratio 11.353, $P < 0.0001$), only age, fasting insulin, and FM maintained their positive correlation with CRP ($P < 0.01$, $P < 0.0001$ and $P = 0.05$, respectively).

Additive multivariate analyses were performed by replacing fasting glucose and insulin with HOMA_{IR} and by includ-

Table 1 C-reactive protein plasma levels and other characteristics of study subjects ($n = 201$)

| Variable | Mean \pm standard error |
|---------------------------------|---------------------------|
| Age (y) | 33.9 \pm 0.82 |
| BMI (kg/m ²) | 33.7 \pm 0.69 |
| Waist circumference (cm) | 101.8 \pm 1.47 |
| Fat mass (kg) | 38.1 \pm 1.36 |
| Fat-free mass (kg) | 49.2 \pm 0.57 |
| Fasting glucose (mmol/l) | 4.56 \pm 0.05 |
| Fasting insulin (μ U/ml) | 20.6 \pm 0.87 |
| HOMA _{IR} | 4.33 \pm 0.22 |
| Total cholesterol (mmol/l) | 4.95 \pm 0.07 |
| HDL-cholesterol (mmol/l) | 1.53 \pm 0.03 |
| Triglycerides (mmol/l) | 1.09 \pm 0.04 |
| Systolic blood pressure (mmHg) | 119.6 \pm 1.08 |
| Diastolic blood pressure (mmHg) | 77.4 \pm 0.72 |
| C-reactive protein (mg/dl) | 0.53 \pm 0.04 |

Table 2 Relationship of C-reactive protein (CRP) plasma levels (mg/dl) with other parameters of study subjects ($n = 201$)

| Variable | r |
|---------------------------------|---------|
| Age (y) | 0.223* |
| BMI (kg/m ²) | 0.300** |
| Waist circumference (cm) | 0.337** |
| Fat mass (kg) | 0.341** |
| Fat-free mass (kg) | 0.259** |
| Fasting glucose (mmol/l) | 0.276** |
| Fasting insulin (μ U/ml) | 0.441** |
| HOMA _{IR} | 0.468** |
| Total cholesterol (mmol/l) | 0.041 |
| HDL-cholesterol (mmol/l) | -0.066 |
| Triglycerides (mmol/l) | 0.111 |
| Systolic blood pressure (mmHg) | 0.039 |
| Diastolic blood pressure (mmHg) | 0.060 |

* $P < 0.001$; ** $P < 0.0001$.

ing alternatively BMI or waist circumference among the independent variables. When HOMA_{IR} was included in the statistical model along with age, BMI, total cholesterol, HDL-cholesterol, triglycerides, FFM, and FM (fitted model: adjusted r^2 0.273, F -ratio 14.624, $P < 0.0001$), HOMA_{IR}, age, and FM maintained their direct association with CRP plasma levels ($P < 0.001$, $P < 0.005$ and $P = 0.05$, respectively). When waist circumference was entered into the regression model after excluding BMI (fitted model: adjusted r^2 0.258, F -ratio 14.939, $P < 0.0001$), HOMA_{IR}, age, and waist circumference, but not FM, maintained their direct association with CRP ($P < 0.01$, $P < 0.005$ and $P < 0.05$, respectively).

Finally, we observed a gradual increase in CRP plasma levels across the quartiles in which the whole population was divided according to HOMA_{IR} levels (F 4.83, $P = 0.003$ for linear trend; Table 3).

Discussion

In this cross-sectional study of apparently healthy adult women, four factors—age, insulin resistance, central fat accumulation and the amount of total body fat—were found to be the most powerful predictors of CRP concentrations. It is worth noting that subjects with major risk factors of cardiovascular disease (CVD), such as smoking, previous history of CVD, presence of diabetes and hypertension, which are associated with higher levels of CRP, were not enrolled in the study. The clinical and pathophysiological relevance of our findings result from a series of epidemiological data.

Even moderately elevated CRP plasma concentrations have been associated with a significant increase in risk of future myocardial infarction, stroke, and peripheral atherosclerosis among apparently healthy middle-aged men and women.²⁻⁴ Therefore, a significant link between baseline elevations of CRP and future risk of cardiovascular events has been elucidated with certainty. In particular, CRP concentrations have been recently demonstrated to be as strong as apolipoprotein B-100 levels and total cholesterol/HDL-cholesterol ratio in predicting the risk of cardiovascular events in women and even stronger than concentrations of total cholesterol, HDL-cholesterol, lipoprotein(a), and homocysteine.¹⁸

Furthermore, accumulation of body fat, particularly in the abdominal region, has several health implications, including cardiovascular health risk.¹⁹⁻²⁵ Previous studies in middle-

Table 3 C-reactive protein (CRP) concentrations (mg/dl) according to HOMA_{IR} levels in study subjects ($n = 201$)

| Variable | Quartile of HOMA _{IR} levels | | | | P-value for trend |
|----------|---------------------------------------|-----------------|-----------------|-----------------|-------------------|
| | 1 | 2 | 3 | 4 | |
| CRP | 0.47 \pm 0.07 | 0.47 \pm 0.07 | 0.63 \pm 0.09 | 0.83 \pm 0.11 | 0.003 |
| Age | 32.7 \pm 1.46 | 35.6 \pm 1.52 | 35.9 \pm 1.62 | 37.7 \pm 1.84 | NS |

aged and elderly subjects have reported a positive association of both BMI^{4,26,27} and waist-to-hip ratio¹⁴ with CRP concentrations. Similarly, white blood cell count has been found to be significantly associated with BMI, percentage body fat, and insulin concentrations.²⁸

In the present study, we have found positive and independent relationships of CRP levels with age, FM, and measures of insulin resistance, such as fasting insulin and HOMA_{IR}. Moreover, when waist circumference was entered in the multivariate analysis, age, HOMA_{IR}, and waist circumference, but not FM, maintained their direct associations with CRP concentrations.

These results have several pathophysiological explanations and implications. First, they are consistent with previous studies demonstrating that age is one of the main determinants of CRP concentrations, since it accounts for about 10% of the variance in CRP levels.⁸

Second, we have reported that FM is directly associated with CRP plasma levels, independent of age, BMI, and fasting concentrations of glucose, insulin, and lipids. It is worth noting that adipose tissue has been reported to be an important source of IL-6 and TNF- α ,^{10,11} cytokines referred to as main regulators of CRP production.^{12,13} Therefore, this biological feature of adipocytes may explain the statistical relationship between FM and CRP, observed in our study.

Third, our findings suggest that central distribution of body fat, as evaluated by waist circumference, and insulin resistance, as measured by fasting insulin or HOMA_{IR}, are stronger predictors of CRP levels than total adiposity, as calculated by bioimpedance analysis or BMI. The significant link between CRP and insulin resistance has been emphasized by analysis of variance, suggesting a progressive increase in CRP concentrations across the quartiles of HOMA_{IR}, irrespective of age. These findings are consistent with the pioneering study of Yudkin *et al*,¹⁴ reporting a positive association of CRP, TNF- α , and IL-6 concentrations with measures of obesity, central fat distribution, and insulin resistance. Indeed, human abdominal visceral adipose tissue has been reported to release more IL-6 compared to subcutaneous adipose tissue,²⁹ thus explaining our result that waist circumference is a stronger predictor of CRP concentrations than total fatness, expressed as BMI or FM. Since the above-mentioned cytokines display an important inhibitory effect on insulin signaling,^{30–32} it might be hypothesized that adipose tissue is responsible for a mild, chronic inflammatory state, as expressed by levels of CRP, IL-6, and TNF- α , which may induce insulin resistance and endothelial dysfunction, thus leading to atherosclerosis, as previously proposed by Yudkin *et al*.¹⁴ Similarly, the Insulin Resistance Atherosclerosis Study reported that CRP concentrations were independently related to insulin resistance, as calculated by frequently sampled intravenous glucose tolerance test (IVGTT).³³

In conclusion, our study of apparently healthy adult women has shown a strong relationship of total body fat, central fat accumulation, and insulin resistance with CRP

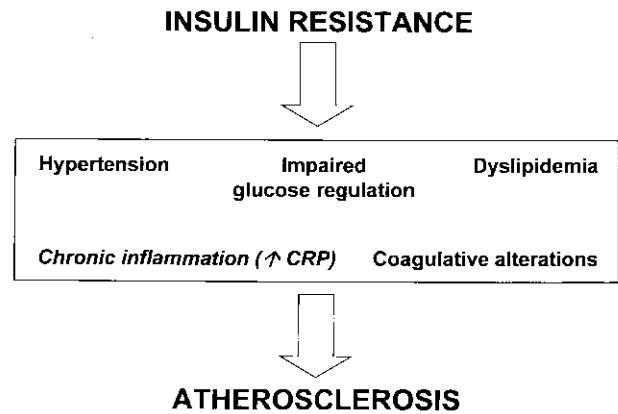


Figure 1 Mild, chronic inflammation: further feature of the metabolic syndrome and mediator of the atherogenic profile of this syndrome.

plasma levels, irrespective of age and other anthropometric and biochemical variables. Since this acute-phase reactant has been shown to be one of the most powerful predictors of risk of cardiovascular events,¹⁸ it can be hypothesized that mild, chronic inflammation may be a further feature of the metabolic syndrome and a mediator of the atherogenic profile of this syndrome (Figure 1).

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