Elevated C-Reactive Protein
Another Component of the Atherothrombotic Profile of Abdominal Obesity

Isabelle Lemieux, Agnès Pascot, Denis Prud’homme, Natalie Alméras, Peter Bogaty, André Nadeau, Jean Bergeron, Jean-Pierre Després

Abstract—Recent studies have suggested that elevated plasma C-reactive protein (CRP) levels are associated with the features of insulin resistance syndrome. In the present study, we have examined the contribution of body composition measured by hydrostatic weighing and of abdominal adipose tissue (AT) accumulation assessed by computed tomography to the variation in plasma CRP levels associated with atherogenic dyslipidemia of the insulin resistance syndrome in a sample of 159 men, aged 22 to 63 years, covering a wide range of adiposity (body mass index values from 21 to 41 kg/m²). Plasma CRP levels showed positive and significant correlations with body fat mass ($r = 0.41$, $P < 0.0001$), waist girth ($r = 0.37$, $P < 0.0001$), and visceral AT accumulation measured by computed tomography at L4 to L5 ($r = 0.28$, $P < 0.0003$). Although CRP levels were associated with plasma insulin levels measured in the fasting state and after a 75-g oral glucose load, no significant correlations were found with plasma lipoprotein levels. Finally, comparison of body fatness, of abdominal fat accumulation, and of the features of the insulin resistance syndrome across quintiles of CRP revealed major differences in body fatness and in indices of abdominal AT accumulation between the lowest and the highest CRP quintiles, whereas no significant differences were found for variables of the plasma lipoprotein-lipid profile. These results suggest that obesity and abdominal AT accumulation are the critical correlates of elevated plasma CRP levels found in men with atherogenic dyslipidemia of the insulin resistance syndrome. (Arterioscler Thromb Vasc Biol. 2001;21:961–967.)

Key Words: C-reactive protein | lipoprotein-lipid profile | glucose-insulin homeostasis | body composition

There is growing recognition that coronary heart disease (CHD) has an inflammatory component. Prospective studies have shown that plasma C-reactive protein (CRP) concentration, a marker of the acute-phase reaction, can predict CHD events in subjects with or without established cardiovascular disease beyond what can be estimated by traditional risk factors.

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The mechanisms underlying elevated plasma CRP levels among subjects at risk for future coronary events are poorly understood. Whether CRP plays a direct role in the multifactorial etiology of CHD or whether it is a marker of endothelial stress or damage or of coronary atherosclerosis is still a matter of debate. Among these possibilities, an elevated plasma CRP concentration is likely to be a marker of the inflammation of the coronary wall associated with a cluster of altered metabolic risk factors.

In this regard, there is increasing evidence that the features of insulin resistance syndrome (namely, abdominal obesity, hyperinsulinemia, high triglyceride [TG]–low HDL cholesterol dyslipidemia, and elevated plasminogen activator inhibitor-1 and fibrinogen concentrations) are all associated with increased CRP levels. Yudkin et al have shown that an increased plasma CRP concentration, a marker of a low level of chronic inflammation, was related to the features of insulin resistance syndrome and to endothelial dysfunction. More recently, Hak et al reported an independent association between waist girth (a crude but useful index of abdominal obesity) and CRP levels, suggesting that the expanded abdominal fat depot (a source of interleukin-6 [IL-6], a potent stimulator of CRP synthesis by the liver) may be an important factor that will help to explain the inflammatory state of the insulin resistance syndrome.

However, studies that have used direct measurements of visceral versus subcutaneous adipose tissue (AT) obtained by imaging techniques such as computed tomography have shown that the visceral AT depot is the critical correlate of the atherothrombotic risk profile of the insulin resistance syndrome. To the best of our knowledge, the potential
associations between visceral AT accumulation and plasma CRP levels have never been examined. Therefore, we explored the relationship between abdominal subcutaneous and visceral AT accumulation and plasma CRP levels and examined their associations with glucose tolerance as well as with plasma insulin and lipoprotein concentrations in a sample of 159 men.

Methods

Subjects
A total of 159 adult men (aged 43.3±7.9 [mean±SD] years) were recruited through the media and selected to cover a wide range of body fatness values (body mass index [BMI] ranged from 21.0 to 41.0 kg/m²). Subjects gave written consent to participate in the present study, which was approved by the Medical Ethics Committee of Laval University. Men with diabetes or CHD were excluded. None of the subjects was on medication known to affect insulin action or plasma lipoprotein-lipid levels. No subject was on an anti-inflammatory drug either before or at the time of the study. Individuals using aspirin as a chronic medication were excluded from the study. Subjects were not allowed to take any medication for at least 24 hours before any metabolic investigation.

Anthropometric Measurements
A hydrostatic weighing technique was used to measure body density, which was obtained from the mean of 6 measurements. Pulmonary residual volume was measured before immersion in the hydrostatic tank, with use of the helium dilution method of Meeney and Kaltenrieder.20 Percent body fat was derived from body density by using the equation of Siri.21 Height, body weight, and waist and hip circumferences were measured according to the procedures recommended at the Airlie Conference,22 and the waist-to-hip ratio was calculated.

Computed Tomography
Visceral AT accumulation was assessed by computed tomography, which was performed on a Somatom DRH scanner (Siemens) by use of a previously described procedure.23 Briefly, each subject was examined while he was in the supine position with both arms stretched above his head. The scan was performed at the abdominal level (between L4 and L5 vertebrae) by use of an abdominal scout radiograph to standardize the position of the scan to the nearest millimeter. Total AT area was calculated by delineating the abdominal scan with a graph pen and then computing the AT surface with an attenuation range of −190 to −30 Hounsfield units.24 The abdominal visceral AT area was measured by drawing a line within the muscle wall surrounding the abdominal cavity. The abdominal subcutaneous AT area was calculated by subtracting the visceral AT area from the total abdominal AT area. The sagittal diameter, a measurement that can be easily obtained from the image of the abdomen generated by the computer, was also determined.

Plasma Lipoprotein-Lipid Variables
Blood samples were collected from an antecubital vein into vacuum tubes containing EDTA after a 12-hour overnight fast for the measurement of plasma lipid and lipoprotein levels. Cholesterol and TG levels were determined in plasma and lipoprotein fractions by use of a Technicon RA-500 (Bayer), and enzymatic reagents were obtained from Randox. Plasma VLDLs (density 1.006 g/mL) were isolated by ultracentrifugation.24 The HDL fraction was obtained after precipitation of LDL in the infranatant (density >1.006 g/mL) with heparin and MnCl₂.25 The cholesterol and TG concentrations of the infranatant were measured before and after the precipitation step. ApoB and apoA-I concentrations were measured in plasma and in the LDL fraction by the rocket immunoelectophoretic method of Laurell,26 as previously described.27 Lyophilized serum for apoB measurements were prepared in our laboratory and calibrated with reference standards obtained from the Centers for Disease Control and Prevention, and the results were validated against external quality controls for apoB (Canadian Reference Laboratory, 1996). The cholesterol content of HDL₃ and HDL₄, subfractions prepared by the precipitation method was also determined.28

Oral Glucose Tolerance Test
A 75-g oral glucose tolerance test was performed in the morning after an overnight fast. Blood samples were collected in EDTA-containing tubes (Miles Pharmaceuticals) through a venous catheter placed in an antecubital vein at −15, 0, 15, 30, 45, 60, 90, 120, 150, and 180 minutes for the determination of plasma glucose and insulin concentrations. Plasma glucose was measured enzymatically,29 whereas plasma insulin was measured by radioimmunoassay with polyethylene glycol separation.30 The total glucose and insulin areas under the curve during the oral glucose tolerance test were determined by the trapezoid method.

Determination of CRP Concentrations
Concentrations of CRP were assessed in deeply frozen plasma samples (−80°C). CRP levels were measured with a highly sensitive immunoassay that used a monoclonal antibody coated with polystyrene particles (hs-CRP); the assay was performed with a Behring BN-100 nephelometer (Dade Behring) according to the methods described by the manufacturer.31 The run-to-run coefficient of variation at CRP concentrations ranging from 1.0 to 10 µg/mL was <5%.

Statistical Analyses
Group differences for continuous variables were examined either by the Student unpaired t test or by the general linear model, and the Duncan post hoc test was used in situations in which a significant group effect was observed. Pearson correlation coefficients were calculated to quantify the univariate associations among variables. Stepwise multiple regression analyses were computed to sort out the contribution of fat mass, waist girth, visceral AT, and fasting insulin levels to the variance of plasma CRP concentrations. All these analyses were performed with the SAS statistical system (SAS Institute).

Results
The sample of men of the present study was characterized by moderately elevated average BMI (30.3±3.9 kg/m²) and waist girth (101.0±9.3 cm) values. Subjects had relatively normal total cholesterol (5.32±0.73 mmol/L) concentrations but presented low HDL cholesterol (0.91±0.18 mmol/L) levels, leading to an elevated cholesterol/HDL cholesterol ratio (6.08±1.34). The average CRP level was 2.21±1.96 µg/mL and ranged from 0.17 to 9.67 µg/mL.

To examine the contribution of overall adiposity to the variation of CRP levels, the entire sample was divided on the basis of BMI values (for nonobese group, BMI <25 kg/m²; for overweight group, BMI 25 to 30 kg/m²; and for obese group, BMI ≥30 kg/m²; Table 1). No difference in age was noted between the 3 BMI groups. The plasma lipoprotein-lipid profile of overweight and obese subjects was significantly different from nonobese individuals, with the exception of apoA-I, cholesterol, LDL cholesterol, and HDL₃ cholesterol levels, which were similar between the 3 groups. Moreover, overweight and obese men were characterized by elevated fasting insulin concentrations, and obese men had the highest CRP levels (1.94±1.92 versus 1.24±1.25 versus 2.86±2.08 µg/mL [P<0.0001] for nonobese, overweight, and obese men, respectively).

Table 2 shows relationships between CRP concentrations and indices of body fatness and of abdominal AT accumulation as well as metabolic risk profile. All anthropometric variables were significantly correlated with plasma CRP levels (0.28<r<0.41, P<0.0003). Among body fatness and
AT distribution indices, total body fat mass was the variable that showed the highest correlation with CRP levels ($r=0.41$, $P<0.0001$). However, associations of plasma lipoprotein variables with CRP levels revealed that there was no relationship between lipoprotein-lipid variables and CRP concentrations. There was also no relationship between CRP and fasting insulin levels reached significance with fat mass showed the highest CRP concentrations ($r=0.41$, $P<0.0001$). There was also no relationship between CRP and fasting glucose concentrations, whereas the association between CRP and fasting insulin levels reached significance ($r=0.17$, $P<0.05$).

Anthropometric indices and lipoprotein-lipid variables were also examined according to CRP quintiles. As shown in Figure 1, progressive increases in BMI, fat mass, visceral AT area, and waist girth were observed across quintiles of CRP concentrations.

We also examined the relationship of CRP concentrations to plasma glucose and insulin levels measured in the fasting state and after a 75-g glucose load. As shown in Figure 2, the response to a glucose load was not different in individuals with different CRP levels. Results presented in Figure 2 indicate that the insulin response to the 75-g glucose load was greater among men in the top CRP quintile compared with men in the first CRP quintile ($P<0.05$).

Multivariate regression analyses were also conducted to sort out the contribution of waist girth, visceral AT area, fat mass, and insulin levels to the variance in CRP concentrations, inasmuch as these variables were all significantly associated with CRP levels. These analyses revealed that after including body fat mass in the model, no other variable examined in the present study further contributed to the variance of CRP levels (16.4%, $P<0.0001$).

Finally, to investigate further the respective contributions of total body fatness and of abdominal AT accumulation to the variance in CRP levels, subjects were divided according to the 50th percentile value of fat mass and waist girth or visceral AT area. As shown in Figure 3, an elevated waist circumference or body fat mass alone was not associated with significant increases in CRP concentrations. However, subjects characterized by elevated waist circumference along with fat mass showed the highest CRP concentrations ($P<0.0001$, Figure 3A). Similar results were obtained when subjects were subgrouped on the basis of visceral AT and body fat mass ($P<0.0001$, Figure 3B).

**Discussion**

There is an emerging consensus that CHD has a multifactorial etiology, including atherosclerotic, prothrombotic, and inflammatory components. Therefore, beyond the assessment...
of conventional CHD risk factors, new markers have been explored in prospective observational studies with the hope that they might improve our ability to predict the risk of developing an acute coronary event.

Among those putative new markers of CHD risk, CRP is a major acute-phase protein associated with chronic systemic inflammation and has been suggested to predict CHD risk beyond traditional risk factors.3–9 CRP has been shown to be associated with the development of CHD events in subjects with3–5 and without6–9 established cardiovascular disease, and case-control prospective studies have suggested that it may be a new independent CHD risk factor.6–8,32 However, its exact role in the etiology of CHD remains obscure. CRP may be a marker of the inflammatory component of the atherosclerotic disease process. It may also be a marker of an as-yet-undefined inflammatory process (eg, chronic infection), which may be favoring the development of atherosclerosis. Finally, CRP might be pathogenic in CHD. For example, it has the ability to induce monocytes to express tissue factors that may favor the occurrence of vascular atherosclerosis.33

The objective of the present study was to examine the relationship between the features of the insulin resistance syndrome and CRP concentrations, with particular attention to abdominal fat accumulation measured by computed tomography. We found significant relationships between plasma CRP levels and all indices of adiposity, such as BMI, total body fat mass, waist girth, sagittal diameter, and subcutaneous and visceral AT areas. These results are consistent with the role of human AT in the regulation of CRP levels.11,12,14,34 In a random sample of 303 men aged 50 to 69 years, Mendall et al34 had reported a significant association between the BMI and CRP levels. A significant relationship of CRP levels with the BMI was also noted among elderly men and women in the Cardiovascular Health Study.11

The reasons for the association between plasma CRP and indices of adiposity are not clear, but several mechanisms may link AT with elevated CRP levels. It has been reported that circulating levels of tumor necrosis factor (TNF)-α are

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<td>Fasting insulin</td>
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**Figure 1.** BMI, body fat mass, visceral AT area, and waist girth according to quintiles of plasma CRP levels. The significant difference (P<0.0001) from the corresponding quintiles is indicated above the standard error.

**Figure 2.** Plasma glucose and insulin concentrations in the fasting state and after the 75-g oral glucose load in the first (open inverted triangles) vs the top (solid inverted triangles) CRP quintiles. Bar charts show plasma glucose as (mmol L−1 · min−1) · 10−3 and insulin total areas as (pmol L−1 · min−1) · 10−3 under the curves. *P<0.05; †P<0.06.
increased in obesity and that TNF-α can stimulate the production of CRP. Furthermore, levels of IL-6, which also induces the production of CRP, have been found to be elevated in obese individuals. Because the synthesis of CRP by the liver is largely regulated by IL-6 and because ~30% of total circulating concentrations of IL-6 originate from AT in healthy subjects, these relationships are compatible with an AT origin of IL-6. Moreover, a recent study has shown that the higher production of IL-6 from AT appears to be more closely related to the increase of total body fat mass than to an overexpression of IL-6 by the AT.

To the best of our knowledge, the present study is the first to examine the contribution of the accumulation of visceral fat (a component of the insulin resistance syndrome) to plasma CRP concentrations. Despite the fact that the amount of total body fat was the best correlate of CRP levels, the highest plasma CRP concentrations were observed among men who had simultaneous elevations in visceral AT accumulation and in total body fatness, whereas individuals with an elevated body fat mass alone had CRP levels that were not significantly different from men with elevated amounts of visceral AT alone. Hak et al recently reported that CRP was strongly related to waist circumference even after adjustment for BMI. Because waist circumference has been shown to be the best anthropometric index to predict visceral AT accumulation, these results suggest that abdominal fat deposition can be an important determinant of an inflammatory metabolic state. However, in the present study, visceral AT failed to make an independent contribution to the variance in CRP levels after control for total body fat mass. This phenomenon could be explained by the strong collinearity of visceral AT and total adiposity indices in the present sample of men. Because the relationship of visceral AT to body fat mass is weaker in women than in men, it will be relevant and interesting to study the correlates of CRP levels in women.

Associations between plasma CRP concentrations and the lipoprotein-lipid profile have been observed in men and women. Elevated plasma CRP levels have been reported among subjects with high TG–low HDL cholesterol dyslipidemia associated with the insulin resistance syndrome. These associations between CRP and lipid values have been reported to persist even after adjustment for BMI. However, the relationships between plasma CRP levels and total cholesterol or LDL cholesterol have been equivocal. In the present study, there was no relationship between CRP and cholesterol, LDL cholesterol, TG, or HDL cholesterol concentrations. We believe that the relative homogeneity of our sample (healthy men covering a wide range of body fatness values) could help to explain this lack of relationship. Furthermore, it may also be possible that relationships reported in other studies between the lipid profile and CRP levels were not causal but largely explained by the concomitant variation in waist circumference.

Relationships between CRP concentrations and fasting insulin levels have been observed in some studies, and this association has been shown to persist after adjustment for BMI. In the present study, we also found a relationship between CRP concentrations and insulinemia (a crude marker of insulin resistance in nondiabetic subjects), suggesting that hyperinsulinemia resulting from insulin resistance is also associated with a state of low chronic inflammation. However, which parameter of the dysmetabolic syndrome is the critical determinant of elevated plasma CRP levels among individuals with insulin resistance syndrome is a question that has not been satisfactorily addressed. The associations between CRP and the cluster of metabolic features of the insulin resistance syndrome, which is characterized by alterations in plasma glucose–insulin homeostasis and in the lipoprotein-lipid profile in the presence of abdominal obesity, could be explained by the action of cytokines on metabolism, whose effects can be modulated by insulin. Indeed, IL-6 can increase hepatic gluconeogenesis and TG synthesis. Moreover, TNF-α, which induces IL-6 synthesis, has been also implicated in the pathogenesis of insulin resistance, and it inhibits lipoprotein lipase activity while stimulating hepatic lipogenesis. Thus, our results and observations suggest that an increased cytokine flux, arising from expanded abdominal AT, could be largely responsible, although not exclusively, for the metabolic abnormalities associated with the features of insulin resistance syndrome, including a state of low chronic inflammation, which would exacerbate CHD risk.
ifiable risk factor is currently uncertain, although recent studies have suggested that several common preventive therapies, such as the use of statins and more recently fibrates, may reduce plasma CRP levels. Additional studies are needed to verify whether weight loss can also reduce the inflammatory state of high-risk abdominally obese men with elevated plasma CRP concentrations.

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