Differentiation Between Obesity and Insulin Resistance in the Association With C-Reactive Protein

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Background—Plasma C-reactive protein (CRP) concentrations are increased in obese and/or hyperinsulinemic individuals. The goal of this study was to determine if the relation between insulin resistance and CRP was independent of obesity.

Methods and Results—Plasma CRP concentrations were measured before and after 3 months of calorie restriction in 38 healthy, obese women. Steady-state plasma glucose (SSPG) concentration during a 180-minute infusion of octreotide, glucose, and insulin was used to stratify participants into insulin-resistant (IR, n = 20) or insulin-sensitive (n = 18) groups, similar in terms of mean age (46±2 versus 44±2 years), body mass index (32.0±0.4 versus 31.4±0.3 kg/m²), and waist circumference (96±2 versus 95±2 cm). Mean CRP (0.39±0.08 versus 0.12±0.03 mg/dL, P=0.003) concentrations were higher in the IR group, as were day-long plasma glucose and insulin responses (P<0.001). There was a significant correlation at baseline between CRP and day-long plasma integrated insulin response (r=0.47, P=0.001) but not between CRP and body mass index (r=0.14) or waist circumference (r=0.10). Weight loss was similar in the two groups (8.7±0.9 versus 8.4±0.8 kg) but was associated with significant (P<0.001) decreases in SSPG and CRP concentrations in the IR group only. Regression analysis showed that SSPG and day-long plasma insulin response were the only significant predictors of CRP concentration.

Conclusions—CRP concentrations are elevated predominantly in obese individuals who are also insulin resistant and fall in parallel with weight loss–associated improvements in insulin resistance. The relation between CRP concentrations and insulin resistance is independent of obesity. (Circulation. 2002;106:2908-2912.)

Key Words: inflammation • insulin • risk factors • obesity • syndrome X

A n association between plasma high-sensitivity C-reactive protein (CRP) concentrations and cardiovascular disease has been noted in both men and women.1,2 A recent meta-analysis3 of 7 prospective epidemiologic studies has provided strong evidence that elevated plasma concentrations of CRP predict coronary heart disease (CHD). Understanding this association is of great importance because it may provide new insight into mechanisms of atherosclerosis or thrombotic events as well as lead to potential new prevention strategies or therapeutic interventions. Subsequent studies have demonstrated that CRP concentrations are significantly related to various measures of body fat,4–8 and in one8 of these studies, weight loss led to a fall in CRP concentrations. However, it is not clear if the relation between obesity and CRP is a direct manifestation of excess adiposity or is due to metabolic changes frequently associated with obesity. For example, the prevalence of resistance to insulin-mediated glucose disposal is increased in obese individuals8 and improves with weight loss.9–12 In this context, at least 3 of the studies reporting an association between obesity and CRP concentrations also described a relation between plasma CRP and fasting insulin concentrations,4,5,7 a surrogate measure of insulin resistance. Moreover, we have previously reported that improving insulin resistance with an insulin-sensitizing agent markedly reduced CRP concentrations in the absence of weight loss.13 One way to determine if insulin resistance is associated with CRP independent of obesity is to take advantage of the fact that obese individuals can be insulin sensitive as well as insulin resistant.9–12 We have used this approach in this study and have compared plasma CRP concentrations as well as more conventional CHD risk factors in obese individuals, stratified at baseline into insulin-sensitive (IS) and insulin-resistant (IR) subjects.

Methods

Subjects included a subset of obese women volunteers from the San Francisco Bay area who had participated in two separate weight loss studies11,12 conducted at Stanford University within the past 3 years to investigate possible influences of insulin resistance on weight loss. The Stanford Human Subjects Committee approved both studies, and all subjects gave written informed consent. In both studies, before and after the period of weight loss, blood was drawn after an overnight fast, plasma and serum were separated, and aliquots were taken for subsequent lipid and lipoprotein measurements or frozen for additional assays. In addition, plasma glucose and insulin
Diagnosis and Classification of Diabetes Mellitus. All volunteers nondiabetic, according to the criteria of the Expert Committee on the intake, as previously described. In each of the studies, insulin-mediated glucose disposal was also quantified by a modification of the insulin suppression test as originally described and validated before and after weight loss. Briefly, subjects were infused for 180 minutes with octreotide (0.27 μg/min), insulin (25 mU/m²/min), and glucose (240 mg/m² min). Blood was drawn at 10-minute intervals from 150 to 180 minutes of the infusion to measure plasma glucose and insulin concentrations, and the mean of these 4 values was used as the steady-state plasma insulin (SSPI) and glucose (SSPG) concentrations for each individual. Because SSPI concentrations were similar in all subjects during these tests, the SSPG concentration provided a direct measure of the ability of insulin to mediate disposal of an infused glucose load; the higher the SSPG concentration, the more insulin-resistant the individual.

In each weight loss study, volunteers were instructed by a certified diettian on calorie-restricted diets calculated to lead to a weight loss of 0.5 kg/wk. The period of weight loss ranged from 2.5 to 3.5 months in duration in the two studies, during which time subjects were seen bimonthly to be weighed and receive dietary advice. At the completion of the weight loss phase, subjects were instructed to increase their caloric intake to a level determined to cease further weight loss and to maintain their new weight. Two weeks later, all measurements performed at baseline were repeated.

From these original studies, we selected a subset of individuals who both met the criteria described below and had serum available for measurement of CRP concentrations that had been carefully frozen at −80°C for 1.5 to 3.0 years. Participants were required to have a body mass index (BMI) between 28 and 36 kg/m² and be nondiabetic, according to the criteria of the Expert Committee on the Diagnosis and Classification of Diabetes Mellitus. All volunteers had been in good general health, as determined by a complete medical history and physical examination, and a normal blood count, chemical screening battery, and urinalysis and subjects were not taking lipid-lowering drugs or regularly using anti-inflammatory medications. Those subjects meeting the general inclusion criteria were separated into IR (SSPG value >160 mg/dL, n = 18) and IS (SSPG value <100 mg/dL, n = 20) groups, on the basis of their SSPG values. These values represent the upper and lower 40th percentiles of insulin resistance as measured in 490 healthy volunteers. Subjects with SSPG concentrations between these two cut-points were excluded from the current analyses. Approximately equal numbers of IR and IS subjects were selected from each of the two weight loss studies, and mean duration of weight loss was similar in both groups.

Serum high-sensitivity CRP was measured with a chemiluminescent assay established for use on a DPC Immulite automatic analyzer (Diagnostics Products Corporation). This assay has a sensitivity of 0.01 mg/dL, intra-assay and interassay CV of <8%, and has been previously demonstrated to perform comparably with the Dade Behring high-sensitivity CRP assay. 

Data Analysis and Statistics

All data are expressed as mean ± SEM. Logarithmically transformed variables was satisfactory for parametric tests. Unpaired and paired Student’s t-tests were used to compare baseline demographic and clinical characteristics of IR versus IS subjects and extent of change within groups, respectively. The Wilcoxon Mann-Whitney test was used to compare CRP at baseline and after weight loss in IR versus IS subjects, and the Wilcoxon rank sum test was used to assess the significance of change in CRP in each group separately. Day-long plasma insulin and glucose concentrations were measured as 9 individual time points during the meal profiles, and the day-long integrated insulin and glucose responses were calculated as the area under the curve (trapezoidal method) over these 8 hours. Repeated-measures ANOVA was used to compare the IR and IS groups with respect to baseline and post-weight loss insulin and glucose concentrations and to assess change in day-long integrated insulin and glucose responses for each group separately. Pearson correlation coefficients were calculated to define the relation between log CRP concentrations and selected variables of interest. Multiple linear regression analysis was conducted to assess independent predictors of log CRP concentration. Adjustments for multiple comparisons (paired t-tests) are between weight loss in IR and IS individuals. Statistical comparisons (paired t tests) are between before and after weight loss values within each insulin resistant or insulin sensitive group.

Results

Baseline demographic and clinical characteristics are shown in Table 1. SSPG concentration was significantly higher, by selection, in the IR group (227±8 versus 76±5 mg/dL, P<0.001). In contrast, no significant differences in age, weight, BMI, waist circumference and lipid levels were present between the two groups. The changes in weight and SSPG concentration in response to the calorie-restricted diets are seen in Figure 1. Weight fell by a comparable amount (8.7±0.9 versus 8.4±0.8 kg) and to a similar final value in

![Figure 1. Mean (±SEM) weight (left) and SSPG concentration (right) before and after weight loss in IR and IS individuals. Statistical comparisons (paired t tests) are between before and after weight loss values within each insulin resistant or sensitive group.](image-url)
both groups. SSPG concentration decreased markedly to 165±12 mg/dL (P<0.001) with weight loss in the IR group, although this was still greater than the post–weight loss value in the IS group (75±5 mg/dL, P<0.001). Not surprisingly, SSPG concentrations, which were not elevated in the IS group (by selection), did not change significantly with weight loss.

Day-long plasma glucose and insulin concentrations for both groups, before and after weight loss, are seen in Figures 2 and 3. Day-long plasma glucose concentrations (Figure 2) were significantly higher at baseline in the IR group than in the IS group (P<0.001). In addition, glucose concentration declined significantly (P<0.001) with weight loss in the IR group, and the day-long response was no longer significantly higher than in the IS group (P=0.29). In contrast, day-long plasma glucose responses were essentially the same in the IS group before and after weight loss.

The IR group had significantly greater day-long plasma insulin concentrations (Figure 3) than the IS group at baseline (P<0.001). Furthermore, although day-long integrated insulin concentrations were significantly lower after weight loss (P=0.01) in the IR group, it should be emphasized that the post–weight loss day-long insulin response in this group was still elevated as compared with the post–weight loss day-long response in the IS group (P<0.001). Finally, day-long insulin concentration curves were similar before and after weight loss in the IS group.

The CRP concentrations of the two groups, before and after weight loss, are shown in Figure 4. Baseline CRP concentrations were significantly higher in the IR group than in the IS group (0.39±0.08 versus 0.12±0.03 mg/dL, P<0.001). Furthermore, whereas plasma CRP concentrations were significantly lower after weight loss in the IR group (P<0.04), there was no decline in CRP concentrations in the IS group. Despite the fall in CRP concentrations with weight loss in the IR group, the post–weight loss CRP concentrations were still higher in the IR group than in the IS group (0.27±0.05 versus 0.11±0.02, P=0.008).

Since SSPG concentrations were not distributed continuously (middle 20% excluded from study), we used the day-long plasma integrated insulin response to compare the relation in the entire population between CRP and estimates of insulin-mediated glucose disposal and obesity. The correlation coefficient between CRP and day-long integrated insulin response is displayed in Figure 5 and demonstrates that the greater the day-long ambient insulin levels, the higher the CRP concentration (r=0.47, P=0.005). In contrast, there was essentially no relation between BMI (r=0.14, P=0.43) or waist circumference (r=0.003, P=0.99) and CRP in this population of overweight/obese individuals (data not shown).

To further define the relation between CRP and the anthropometric and metabolic variables quantified in this study, multiple regression analysis was performed at baseline with log CRP as the dependent variable. These results are given in Table 2 and show that only SSPG concentration...
Discussion

It is apparent from the data presented that not all obese individuals had high CRP concentrations. Instead, CRP concentrations in the insulin-sensitive, obese individuals were uniformly low, and elevated CRP concentrations were confined to those obese individuals who were also insulin resistant and hyperinsulinemic. Since the two groups of obese individuals were similar in terms of BMI and abdominal circumference, it seems likely that the higher CRP concentrations in the insulin-resistant subgroup were not due to differences in adiposity. It should be emphasized that our results are not in conflict with previous findings of a relation between adiposity and CRP concentrations. The prevalence of insulin resistance is increased in obese individuals, and the fact that CRP concentration correlated with degree of adiposity in previous studies is consistent with our findings of higher CRP concentrations in insulin resistant individuals. However, since not all obese individuals are insulin resistant, our study design permitted us to demonstrate that CRP concentrations are related to degree of insulin resistance/hyperinsulinemia, independent of either BMI or abdominal circumference. On the other hand, the truncated nature of the values of BMI and waist circumference in our population, and the fact that we only used two estimates of adiposity in our analysis, does not permit us to exclude the possibility that differences in obesity that we did not measure might also modulate CRP concentrations.

In addition to extending understanding of the relation between adiposity and CRP concentrations, our results also provide insight into the previous observation that CRP concentrations fall when obese individuals lose weight. As seen in Figure 3, CRP concentrations only fell in obese individuals who were insulin resistant and hyperinsulinemic at the outset. The fact that CRP concentrations were still higher in the IR group than in the IS group after weight loss may be related to their residual insulin resistance. Although it is also possible that both CRP and SSPG concentrations may have changed further in these IR subjects if the period of weight stability had been longer in duration, there is little published data in this area to permit speculation. The absence of a decline of CRP concentrations in the IS group was not due to insufficient sensitivity of the assay, as the threshold of detection is nearly a log lower than the mean values in the IS group. Thus, it appears that not only are elevations in plasma CRP concentrations confined to those obese individuals who are also insulin resistant, decreases in CRP concentration with weight loss are limited to this group. Both of these results suggest that variations in CRP are modulated by changes in insulin resistance and/or compensatory hyperinsulinemia. Additional support for this interpretation is provided by the results in Figure 5, demonstrating that there was a significant correlation at baseline between the day-long integrated plasma insulin response and plasma CRP concentration in the entire population. The observation that CRP concentrations are strongly related to day-long integrated response is consistent with the results of previous studies that described an association between CRP and fasting plasma insulin concentration. Plasma insulin concentrations are significantly related to insulin-mediated glucose-disposal and can serve as a surrogate measure of insulin resistance. The close relation between insulin resistance and compensatory hyperinsulinemia, coupled with the results of the multiple regression analysis showing that the only independent predictors of CRP concentration were either SSPG concentration or day-long integrated insulin response, makes it difficult to decide whether insulin resistance or hyperinsulinemia is most closely related to CRP concentration.

Finally, although our results document an association between insulin resistance and plasma CRP concentrations, they do not provide insight into the nature of this relation. For example, it has been suggested that heightened inflammatory responses could lead to insulin resistance and compensatory hyperinsulinemia. This has been attributed in large part to the important role of inflammatory cytokines released from adipocytes, a process presumably made more likely with increased adiposity. Alternatively, metabolic abnormalities associated with insulin resistance, including hyperglycemia, elevated free fatty acids, dyslipidemia, and endothelial dysfunction, may induce cell activation and inflammatory responses. Moreover, insulin-resistant subjects appear to demonstrate an excess risk for the development of atherosclerosis, a condition that is in large part an inflammatory process. Therefore, insulin resistance could contribute to higher CRP concentrations. Finally, higher SSPG (ie, greater insulin resistance) and CRP concentrations could both result from a more fundamental and shared abnormality, similar to

<table>
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*Day-long integrated insulin response values were excluded from this model because of the strong correlation with SSPG.
the “common soil” hypothesis suggested by Stern to explain the relation between diabetes and CHD. The results of the current study do not permit us to choose between these various alternatives. However, we have now demonstrated that two separate methods of reducing insulin resistance, for example, through the use of insulin-sensitizing agents or by inducing weight loss, appear to decrease CRP concentrations. Importantly, in both instances, the reductions in CRP were independent of weight loss. Moreover, the current study demonstrates that among individuals with similar weight, BMI, and waist circumferences, CRP concentrations were elevated only in those with insulin resistance. These data would appear to lessen the likelihood that adipocyte-associated inflammatory activity is responsible for modulation of CRP levels in these instances. Although possible, it seems unlikely that these two different, short-duration approaches to reducing insulin resistance would both have the ability to reverse a more fundamental abnormality responsible for both inflammation and insulin resistance. It is therefore tempting to speculate that the higher CRP concentrations present in the obese individuals with high SSPG in this study and the decline observed with weight loss are directly related to the extent of insulin resistance and/or compensatory hyperinsulinemia. Obviously, the appropriateness of this view awaits further study.

Acknowledgments

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