Independent Association Between Plasma Leptin and C-Reactive Protein in Healthy Humans

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Background—C-reactive protein (CRP) is synthesized from the liver and is regulated by cytokines, especially interleukin-6. Leptin, the adipocyte-derived protein product of the ob gene, is related to amount of body fat. The long form of the leptin receptor resembles cytokine receptors, which include the interleukin-6 receptor. Both leptin and CRP may increase in women, in obesity, and in inflammation, and both have been linked to cardiovascular pathophysiological processes and increased cardiovascular risk. We tested the hypothesis that leptin is associated with CRP levels independently of the influences of gender, body mass index (BMI), waist-to-hip ratio, and other variables.

Methods and Results—We studied 100 healthy volunteers (48 men, and 52 women). For all subjects, leptin was independently associated with CRP after adjustment for age, gender, BMI, waist-to-hip ratio, smoking, and alcohol consumption ($F=12.39, P=0.0007$). There was a strong and significant positive relationship between leptin and CRP in both women ($R=0.61, P<0.0001$) and men ($R=0.55, P<0.0001$) considered separately. The association between leptin and CRP was significant even after adjustment for age, BMI, waist-to-hip ratio, smoking, and alcohol consumption in women ($F=7.13, P=0.01$) and men ($F=5.69, P=0.02$). When only subjects with BMI $<25$ kg/m$^2$ were considered (n=47), CRP was not linked to BMI ($R=0.02, P=0.96$), but a significant association between leptin and CRP was still evident ($R=0.55, P<0.0001$).

Conclusions—Leptin and CRP levels are independently associated in normal humans, providing further evidence linking metabolic and inflammatory cardiovascular disease mechanisms. (Circulation. 2004;109:2181-2185.)

Key Words: C-reactive protein ■ cardiovascular diseases ■ inflammation ■ leptin ■ obesity ■ risk factors

Inflammation may contribute importantly to cardiovascular disease, particularly ischemic heart disease and heart failure.\(^1\) C-reactive protein (CRP), a marker of inflammation, is emerging as an important indicator of cardiovascular risk\(^2\)–\(^4\) and is associated with future cardiovascular events in patients with acute coronary artery disease, stable angina pectoris, and history of myocardial infarction.\(^5\) Elevated CRP is also a strong predictor of cardiovascular risk in apparently healthy men and women.\(^1\) CRP may itself contribute to vascular disease by inhibiting nitric oxide synthase,\(^6\) increasing cell adhesion molecule expression,\(^7\) increasing plasminogen activator inhibitor-1,\(^8\) activating vascular smooth muscle cells,\(^9\) and eliciting direct proinflammatory effects on endothelial cells.\(^10\)

Leptin, the adipocyte-derived protein product of the ob gene, is involved in appetite regulation and obesity through central effects at the hypothalamus.\(^11\) Leptin is related to amount of body fat.\(^12\) Leptin is also associated with increased heart rate,\(^13\) blood pressure,\(^13\) and sympathetic neural activity\(^14\) and may contribute to platelet aggregation.\(^15\),\(^16\) Recent data have implicated leptin as an independent risk factor for cardiovascular diseases\(^17\)–\(^19\) even after adjustment for traditional risk factors.\(^20\)

CRP is synthesized by the liver and regulated by cytokines, especially interleukin (IL)-6.\(^21\) The long form of the leptin receptor resembles the gp120 family of cytokine receptors, which includes the IL-6 receptor.\(^11,22\) Leptin also activates Janus kinases and certain signal transducers and activators of transcription, downstream components in the signaling pathways of several proinflammatory cytokines. Other important intracellular pathways related to possible proinflammatory effects of leptin are MAPK (ERK1/2), p38, and nuclear factor-kappaB.\(^23,24\)

Both leptin and CRP may be increased in women, in obesity,\(^12\) and in inflammation,\(^25\)–\(^27\) and both have been linked to cardiovascular pathophysiological processes and increased cardiovascular risk. There is little information on any interaction between leptin and CRP, particularly in

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healthy normal subjects. We tested the hypothesis that plasma leptin is associated with CRP independently of the influences of gender, body mass index (BMI), waist-to-hip ratio, and other variables.

Methods
We studied 100 healthy volunteers (48 men, 52 women). All subjects were free of any acute or chronic disease conditions, including sleep disorders, and on no medications. Occult obstructive sleep apnea was excluded by complete overnight polysomnography in all subjects >25 years of age and/or with a BMI >22 kg/m^2; thus, of all 100 subjects, 83 underwent overnight polysomnography. Baseline demographic data, body fat, BMI, heart rate, blood pressure (Dinamap MPS, Critikon), and venous blood were collected between 8 and 10 AM. Leptin and high-sensitivity CRP levels were measured with radioimmunoassay kits (leptin, Linco Research Inc; CRP, Kamiya Biomedical Corp). Written informed consent was obtained from all subjects. The institutional Human Subjects Review Committee approved the study.

Statistical Analysis
Data are expressed as mean±SEM or median and range. Categorical variables were compared by use of a χ² test. Group comparisons were performed by an unpaired Student’s t test or the Wilcoxon rank-sum test. Because of the positive skewed distribution of leptin levels (Figure 1), subjects were divided according to the quartiles of leptin levels, with increased levels of leptin accompanied by increased CRP levels (Figure 2).

There were positive correlations (Table 1) between BMI and CRP (R=0.47, P<0.0001) and between BMI and leptin (R=0.58, P<0.0001). In bivariate analysis, there was a strong and significant positive relationship between leptin and CRP levels (R=0.64, P<0.0001). In multivariate analysis, leptin was independently associated with CRP after adjustment for age, gender, BMI, waist-to-hip ratio, smoking, and alcohol consumption (F=12.39, P=0.0007). When only subjects with BMI <25 kg/m^2 were considered (n=47), CRP was not linked to BMI (R=0.02, P=0.96), but a significant association between leptin and CRP was still evident (R=0.55, P<0.0001; Figure 3).

Men and women were further considered separately, and the characteristics of each group are described in the Table 2. CRP (0.33±0.05 versus 0.08±0.01 mg/dL, P<0.0002) and leptin (17±2 versus 6±1 ng/mL, P<0.0001) levels were significantly higher in women than men. Significant positive relationships between BMI and CRP (R=0.51, P<0.001 for women; R=0.44, P<0.001 for men) and between BMI and leptin (R=0.75, P<0.0001 for women; R=0.58, P<0.0001 for men) were evident in both women and men. There was a strong and significant positive relationship between leptin and CRP in both women (R=0.61, P<0.0001) and men (R=0.55, P<0.0001). The association between leptin and CRP was significant even after adjustment for age, BMI, waist-to-hip ratio, smoking, and alcohol consumption in both women (F=7.13, P=0.01) and men (F=5.69, P=0.02).

Discussion
The novel finding of the present study is the independent association between leptin and CRP in healthy humans. Although both leptin and CRP levels were significantly higher in women, there was an independent association

| Variables         | Log Leptin |       | Correlation Coefficient | P   | | Correlation Coefficient | P   |
|-------------------|------------|-------|-------------------------|-----| |-------------------------|-----|
| Age, y            | 0.25       | 0.02  | 0.23                    | 0.02|
| BMI, kg/m²        | 0.58       | <0.0001| 0.47                    | <0.0001|
| Waist-to-hip ratio| 0.06       | 0.56  | 0.09                    | 0.37|
| Mean BP, mmHg     | −0.05      | 0.67  | 0.07                    | 0.51|
| Alcohol, drinks/wk| −0.15      | 0.14  | −0.02                   | 0.81|

*BP indicates blood pressure.*
between leptin and CRP in both men and women considered separately. It is unlikely that the leptin–CRP association we describe is secondary to BMI because the relationship was clear and significant even after adjustment for BMI. Furthermore, in subjects with BMI <25 kg/m², CRP was significantly linked to leptin but not to BMI. Thus, the leptin–CRP association cannot be secondary to any common influence of BMI on each of these measures.

The mechanisms linking leptin and CRP are not clear. Adipose tissue is the source of circulating leptin. CRP is synthesized by the liver, largely under the regulation of the proinflammatory cytokines, primarily IL-6. Other proinflammatory cytokines such as IL-1 and tumor necrosis factor-α (TNF-α) may contribute to hepatic synthesis of CRP. Adipocytes are important sources of circulating IL-6 and express TNF-α. Hence, adipocytes, by serving as a common source for both leptin and inflammatory cytokines contributing to CRP synthesis, may explain in part the interaction between CRP levels and leptin. Moreover, IL-1, IL-6, and TNF-α, which contribute to increases in CRP, may act directly on fat cells to increase leptin secretion in settings of acute inflammation. Further supporting adipose tissue as a potential common pathway contributing to the leptin–CRP interaction. Nevertheless, the strong relationship between leptin and CRP in the present study was independent of several measures of adiposity (eg, BMI and waist-to-hip ratio).

Thus, another possibility may be that leptin, either directly or indirectly through the immune system, may alter CRP levels. First, leptin can actually induce the production of various cytokines, including IL-6. Second, IL-6 induces CRP. Third, the leptin receptor has been shown to have signaling capabilities of IL-6-type cytokine receptors. It is conceivable then that leptin may act via IL-6, or perhaps even via the leptin receptor, to upregulate CRP production.

Leptin also has an important role in modulating the immune response. Subcutaneous injection of recombinant leptin in humans consistently produces inflammation at the site of injection, suggesting an immunomodulatory role of leptin. Exogenous leptin upregulates both phagocytosis and the production of inflammatory cytokines and thus regulates proinflammatory immune responses. Leptin also promotes and sustains immune responses mediated by Th1 CD+4 lymphocytes. Surges of leptin have been observed at the onset of experimental autoimmune encephalomyelitis, and these leptin surges correlate with the development of pathogenic T-cell responses. Reduced leptin levels are related to impairment of immune function, characterized by lymphoid atrophy and T-cell dysfunction; these may be restored by administration of exogenous leptin. Therefore, there is compelling evidence implicating leptin as an important modulator of the inflammatory process. Our data now raise the possibility that leptin may also conceivably act to increase levels of CRP, even in healthy normal subjects. If leptin does indeed induce CRP, this may be a mechanism whereby leptin would increase cardiovascular risk.

The clinical significance of the positive and independent relationship that we describe between leptin and CRP is that higher levels of one of these measures of inflammation/cari-

![Figure 3. Correlations between log leptin and log CRP in normal-weight (BMI <25 kg/m²) and overweight/obese (BMI ≥25 kg/m²) subjects. Significant positive relationships are observed in both groups even though CRP did not correlate with BMI in normal-weight subjects, suggesting that leptin–CRP interaction is not explained by effects of BMI.](http://circ.ahajournals.org/content/113/6/2183/F1.large.jpg)

**TABLE 2. Subject Characteristics**

<table>
<thead>
<tr>
<th></th>
<th>Women (n=52)</th>
<th>Men (n=48)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>36±2</td>
<td>36±2</td>
<td>NS</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>Median (range) 25 (19.1–44.4) 26 (18.7–37.2)</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Mean±SEM</td>
<td>27.1±0.9</td>
<td>26.7±0.6</td>
</tr>
<tr>
<td>Waist, cm</td>
<td>88±3</td>
<td>93±2</td>
<td>NS</td>
</tr>
<tr>
<td>Hip, cm</td>
<td>105±2</td>
<td>102±1</td>
<td>NS</td>
</tr>
<tr>
<td>Waist-to-hip ratio</td>
<td>0.83±0.01</td>
<td>0.909±0.01</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Systolic BP, mm Hg</td>
<td>117±2</td>
<td>128±2</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Diastolic BP, mm Hg</td>
<td>69±1</td>
<td>72±1</td>
<td>NS</td>
</tr>
<tr>
<td>Mean BP, mm Hg</td>
<td>85±1</td>
<td>90±1</td>
<td>0.01</td>
</tr>
<tr>
<td>Heart rate, bpm</td>
<td>72±2</td>
<td>67±2</td>
<td>0.04</td>
</tr>
<tr>
<td>Nonsmokers:smokers</td>
<td>44:8</td>
<td>44:4</td>
<td>NS</td>
</tr>
<tr>
<td>Alcohol, drinks/wk</td>
<td>1.19±0.21</td>
<td>2.17±0.34</td>
<td>0.02</td>
</tr>
<tr>
<td>CRP, mg/dL</td>
<td>Median (range) 0.1735 (0.023–1.5) 0.082 (0.021–0.466)</td>
<td>0.0002</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Mean±SEM</td>
<td>0.307±0.046</td>
<td>0.106±0.012</td>
</tr>
<tr>
<td>Leptin, ng/mL</td>
<td>Median (range) 12.5 (2.4–61.1) 4.7 (1.1–11.9)</td>
<td>&lt;0.0001</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Mean±SEM</td>
<td>16.9±2</td>
<td>5.5±0.5</td>
</tr>
</tbody>
</table>

BP indicates blood pressure. P, women vs men.
diovascular risk are linked to elevation in the other. However, both leptin and CRP each act directly and independently through a number of pathophysiological mechanisms to increase cardiovascular risk. Leptin stimulates vascular smooth muscle proliferation,\(^4\) accelerates vascular calcification,\(^4,5\) induces oxidative stress in endothelial cells\(^6,7\) that may contribute to atherogenesis, and promotes coagulation by increasing platelet adhesiveness.\(^8,9\) CRP promotes secretion of inflammatory mediators by vascular endothelium,\(^7\) increases cell adhesion molecule expression,\(^10\) opsonizes LDL for uptake by macrophages in atherosclerotic plaque,\(^11\) decreases endothelial nitric oxide synthase expression and activity,\(^8,9\) and activates vascular smooth muscle cells.\(^9\) With few exceptions,\(^20\) studies examining the cardiovascular risk associated with CRP have not considered the potential additional risk attributable to increased leptin levels.

Furthermore, conditions in which both leptin and CRP may be increased simultaneously, such as obesity and obstructive sleep apnea,\(^29\) may be associated with additive or perhaps synergistic increases in cardiovascular risk. However, whether the pathophysiological processes induced by leptin or CRP are enhanced or redundant when both are elevated is unknown.

The strengths of the present study are the exclusion of occult obstructive sleep apnea and the absence of disease and drug therapy in our subject population, suggesting that the association we describe is physiological. Our data further suggest that any such interaction may be active very early, even before any evidence of overt cardiovascular disease.

In summary, we have demonstrated in healthy humans that increased leptin is associated with increased CRP independently of gender, measures of adiposity, and other variables. Both leptin and CRP have been linked to inflammatory and cardiovascular disease processes. These data provide further evidence linking the metabolic and inflammatory mediators of cardiovascular disease processes.

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