Role of C Reactive Protein (CRP) in Leptin Resistance

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Abstract: Increased plasma levels of both leptin and C reactive protein (CRP) have been reported in a number of conditions, including obesity, and have been linked to cardiovascular pathophysiological processes and increased cardiovascular risk; interestingly these two biomarkers appear to be able to reciprocally regulate their bioavailability, through complex mechanisms that have not been completely clarified yet. Here we first review clinical evidence suggesting not only that the circulatory levels of CRP and leptin show an independent correlation, but also that assessing them in tandem may result in an increased ability to predict cardiovascular disease. We summarize also molecular studies showing that leptin is able to promote CRP production from hepatocytes and endothelial cells in vitro and discuss the studies addressing the possibility that in vivo leptin administration may be able to modulate plasma CRP levels. Furthermore, we describe two studies demonstrating that CRP directly binds leptin in extra-cellular settings, thus impairing its biological actions. Finally we report genetic evidence that common variations at the leptin receptor locus are associated with CRP blood levels. Overall, the data reviewed here show that the chronic elevation of CRP observed in obese subjects may worsen leptin resistance, contributing to the pathogenesis of cardiovascular disease, and highlight a potential link between conditions, such as leptin resistance and endothelial dysfunction, that may be amenable of pharmacological treatment targeted to the disruption of leptin-CRP interaction.

Keywords: Leptin, CRP, cardiovascular disease, leptin resistance.

INTRODUCTION

The role of the adipokine leptin in the regulation of energy balance, has been firmly established since its discovery almost twenty years ago [1]. Animal models lacking either the leptin gene (ob/ob mice) or the gene encoding for its membrane receptor (db/db mice) are extremely obese, hyperinsulinemic and insulin resistant. More importantly, it has been demonstrated that reestablishing leptin signaling in these mice normalizes body weight as well as all metabolic and endocrine alterations [2,3]. Soon after the discovery of this adipokine, it was therefore hypothesized that a reduction in leptin levels and/or an impairment in its secretion would be responsible also for human obesity. However, while leptin deficiency has indeed been shown to cause rare forms of severe human obesity [4], the majority of obese individuals show markedly increased plasma leptin concentrations, reflecting the greater amount of adipose tissue [5]. This excess of circulating leptin results in a state of resistance, characterized by a reduced response to the hormone action. In fact, hyperleptinemia could be considered a marker of leptin resistance and is not only commonly observed in obese subjects, but also independently associated with insulin resistance and cardiovascular disease (CVD) in humans [6]. Interestingly, several studies have shown an independent relationship between high leptin and atherosclerosis, myocardial infarction, stroke, and coronary artery intima-media thickness, suggesting that high leptin levels are associated with increased cardiovascular risk [7].

Mechanistically, it has been demonstrated that leptin has a role in several processes relevant to cardiovascular disease, including the regulation of arterial pressure and vascular function and that there is a significant cross-talk between leptin and insulin signaling pathways [8]. These important mechanisms have been reviewed elsewhere, whereas in this Review we will focus on the multi-faceted cross talk between leptin and the pro-inflammatory molecule C reactive protein (CRP).

CRP is an acute-phase protein produced mainly by hepatocytes, which belongs to the family of pentaxins and as such consists of five identical non-covalently linked subunits. CRP concentration increases 4 to 6 hours after acute tissue injury or inflammation and declines rapidly with the resolution of the inflammatory process [9]. Conversely, low-grade chronic inflammation, a condition underlyrng insulin resistance and associated with cardiovascular disease and type 2 diabetes [10], produces minor elevations of CRP in the 3- to 10-mg/L range. Epidemiological evidence indicates that elevated CRP levels predict the development of type 2 diabetes and glucose disorders [11-13]. In addition, several cross-sectional studies in non-diabetic subjects, in the general population or in individuals with impaired glucose tolerance (IGT)/impaired fasting glucose (IFG) have confirmed that acute-phase reactants, such as CRP, are positively correlated with measures of insulin resistance, adiposity, and circulating triglyceride and negatively correlated with HDL cholesterol concentrations [14-18]. Furthermore in vivo studies have shown that, in addition to being a sensitive marker of inflammation, CRP has direct proinflammatory effects. In endothelial cells, CRP decreases nitric oxide and prostacyclin release and increases the expression levels of monocyte chemoattractant protein-1, interleukin-8, and plasminogen activator inhibitor-1. In monocye-macrophages, CRP induces tissue factor secretion, increases reactive oxygen species and proinflammatory cytokine release, promotes monocyte chemotaxis and adhesion, and increases oxidized low-density lipoprotein uptake. Also, CRP has been shown in vascular smooth muscle cells to increase inducible nitric oxide production, increase NF-kB and mitogen-activated protein kinase activities, and, most importantly, up-regulate angiotensin type-1 receptor resulting in increased reactive oxygen species and vascular smooth muscle cell proliferation [19-21]. More recently, it has also been reported that CRP has a direct inhibitory effect on insulin signaling and action in a skeletal muscle cell model [22].

PLASMA LEPTIN AND CRP LEVELS SHOW A DIRECT CORRELATION

Increased plasma levels of both leptin and CRP have been reported in a number of conditions, including obesity and inflammation, and have been linked to cardiovascular pathophysiological processes and increased cardiovascular risk [9,11-18,23-26]. Several studies have demonstrated that a direct correlation exists between the concentrations of the two biomarkers (Table I). A first, cross-sectional study on 179 apparently healthy Japanese male col-
Table 1. Clinical studies showing a direct association between CRP and leptin plasma levels.

| Subjects (N and description) | Age     | CRP (mg/L) Mean±SD or range | Leptin (ng/ml) Mean±SD or median | Statistical Correlations |
|------------------------------|---------|-----------------------------|----------------------------------|-------------------------|
| 179 healthy volunteers       | 18-22   | 0.51±1.45                   | 2.3±2.9                          | R=0.28, P<0.0002        |
| 100 healthy volunteers      | 36±2    | 3.07±0.046 W 1.06±0.012 M    | 16.9±2 W 5.5±0.5 M               | R=0.61, P<0.0001 W R=0.55, P<0.0001 M |
| 946 healthy community-dwelling, older adults | 65-102 | 2.8 (4.4)                   | 8.6 (12.3)                        | β = 0.20, P <0.0001     |
| 1862 healthy young adults   | 24-39   | 0.75 (0.32-1.93) W 0.56 (0.29-1.27) M | 12.5 (7.8-19.5) W 4.1 (2.4-6.5) M | R=0.47, P<0.0001 W R=0.46, P<0.0001 M |
| 20 OB                       | 47.8±13.3 OB | 5.7±3.4 OB                 | 17.6±8.7 OB                      | R=0.43, P<0.044 #       |
| 5 NO                        | 56.8±7.6 NO | 3.8±1.6 NO                 | 6.2±3.3 NO                       | R=0.53, P<0.001 #       |
| 63 (28 MO)                  | 35±6.9  | 8.2 (0.13-56.6)             | 52.7±19.8 MO 7.6±4.8             | R=0.330, P<0.001        |
| 148 with T2D                 | 37-84   | 0.78±0.049                  | 5.6±0.4                          | R=0.32, P<0.0001 W R=0.22, P<0.0001 M |
| 150 with T2D                 | 52.8±11.1 W 51.9±9.9 M | 2.34±1.65 1.72±2.1          | 9.82±6.78 4.76±2.44           | β= 0.326, P = 0.01      |
| 6251 (598 with T2D)         | 44.4±0.21 § | NA                         | NA                               | R=0.41, P<0.001 W R=0.29, P<0.001 M |
| 1460 (894 with T2D)         | 30-77   | 1.7 (0.8-3.8)               | 12.1 (6.5-23.2)                  |                          |

#The correlations were not longer significant after adjustment for BMI in obese individuals; § values are expressed as mean ±SE.

Similar findings have been reported in obese individuals [31,32], even if the results from these studies indicate that, in this group of subjects, the relationship between leptin and CRP may be a reflection of fat mass, since the correlation between the two variables was abolished when the data were adjusted for BMI. The independent correlation between leptin and CRP levels has also been investigated in subjects with type 2 diabetes. In a study comprising 148 Japanese subjects with type 2 diabetes, serum CRP levels were positively correlated with leptin (R=0.330, P<0.001), and leptin was shown, by multiple regression analysis, to be an independent predictor of CRP concentration (R=0.330, P<0.001), along with interleukin-6 (IL-6) and triglycerides plasma levels [33]. Similar results were reported by a study carried out in 150 recently diagnosed type 2 diabetic patients showing a significant association between serum CRP and leptin (β=0.326, P=0.01), after adjustment for age and gender [34].

Table 2. Clinical studies showing a direct association between CRP and leptin plasma levels.

leage students aged 18 to 22 reported that CRP serum levels had a positive correlation with leptin levels (R=0.28, P<0.0002), which was independent from body mass index (BMI) [27]. This finding was confirmed by a subsequent study, carried out in a cohort of 100 healthy volunteers (48 men, and 52 women). In this study, it has been observed that leptin was independently associated with CRP after adjustment for age, gender, BMI, waist-to-hip ratio, smoking, and alcohol consumption (P<0.0007). Furthermore, a significant relationship between leptin and CRP was reported in both sexes, when men (R=0.55, P<0.0001) and women (R=0.61, P<0.0001) were analyzed separately. Importantly, the association between leptin and CRP remained significant when the analysis was restricted to lean individuals (BMI <25 kg/m²) (R=0.55, P<0.0001) [28].

An independent association between leptin and CRP levels (β coefficient=0.29, P<0.0001) was found in a study carried out in 946 community-dwelling older subjects (398 men, 548 women; age range 65 to 102 years) [29]. The independent association between leptin and CRP in healthy individuals was also confirmed by a larger population-based study conducted at five health centers in Finland comprising 1862 young adults (971 women; 891 men) aged 24-39 yr [30]. This study reported that CRP and leptin levels were significantly correlated (R=0.47, P<0.0001 for women; R=0.46, P<0.0001 for men). In multiple regression analyses including age, BMI, waist circumference, insulin, lipids, systolic and diastolic blood pressure, smoking status, and use of oral contraceptives in women, leptin proved to be the main determinant of CRP in men (P<0.0001) and the second most important determinant in women (P<0.0001).
some studies [36,37] and suggest that the two markers should be considered in tandem when estimating CVD risk [35]. This suggestion is supported by the results of a more recent study aimed to test the hypothesis that CRP levels may modify the relationship of leptin concentration with coronary artery calcium (CAC), a measure of coronary atherosclerosis in individuals with excess adiposity. The authors examined 1,460 asymptomatic individuals from two community-based cross-sectional studies, the Study of Inherited Risk of Coronary Atherosclerosis (SIRCA) and the Penn Diabetes Heart Study (PDHS), coordinated at a single, university-based research center at the University of Pennsylvania [38], and analyzed the interaction of log-transformed plasma leptin levels with higher CRP levels. The association of plasma leptin with CAC was modified by higher CRP regardless of the cut-point used (interaction term $P$ values all <0.01 in fully adjusted models), while no interaction with CRP was observed in control analyses with adiponectin, BMI or waist circumference. The authors concluded that CRP itself, or the inflammatory pathways that it captures, affects the relationship of circulating leptin concentration to the burden of underlying atherosclerosis in overweight and obese individuals, clinical conditions where chronic inflammation and leptin resistance coexist.

MOLECULAR MECHANISMS LINKING LEPTIN AND CRP: LEPTIN IS ABLE TO REGULATE CRP EXPRESSION

The physiological and molecular mechanisms that link leptin and CRP appear complex (Fig. 1). Leptin is produced by the adipose tissue, and adipocytes are also an important source of circulating inflammatory cytokines, such as IL-6, which in turn promote CRP synthesis [39]. However, leptin itself may be able to stimulate CRP synthesis from the liver, as suggested by experiments performed in primary human hepatocytes. In fact when human hepatocytes were incubated with human leptin at physiological concentrations (1-16 nM) for 24 h a dose-dependent accumulation of secreted CRP in the culture medium was observed; interestingly preincubation with a specific Phosphatidylinositol 3-kinases (PI3K) inhibitor completely blocked this leptin action, suggesting that leptin-induced hepatic production of CRP is a PI3K-dependent process. To exclude the possibility that they were observing a IL-6 mediated effect, the authors incubated primary hepatocytes in the presence of IL-6 at doses comparable to those observed in vivo in obese individuals and showed that, at these concentrations, IL-6 was unable to promote CRP synthesis [40]. Subsequently, following the accumulating evidence indicating that CRP can be produced not only from hepatocytes, but also from additional cell types [41], similar results have been obtained by two independent studies in endothelial cells [42,43]. A first study showed that in human artery coronary endothelial cells (HAECs) a dose dependent increase of CRP levels was observed with increasing concentration of leptin (0 to 400 ng/ml) [42]. Interestingly, the increase of CRP expression was attenuated in the presence of anti-leptin receptor antibodies, indicating that a classical leptin signaling was mediating this leptin effect. A subsequent study confirmed these findings, showing that lower, and more physiological, leptin concentrations (5-10 ng/ml) were able not only to induce CRP synthesis, but also to promote its release in the culture medium [43].

These in vitro data are supported by in vivo experiments showing that exogenous leptin administration is able to increase plasma CRP concentration. In a first single-blind, 22-day, placebo/drug/placebo study, six subjects received recombinant methionyl human leptin (r-metHuLeptin) at the dose of 0.3 mg/kilogram subcutaneously for 6 days. No demonstrable effect of leptin administration on energy metabolism was evident in this small group of never-obese individuals; leptin treatment induced an elevation in CRP, IL-6, and haptoglobin, even if the small number of patients studied made impossible to determine whether these changes were related to leptin itself, to the interaction of leptin with a preexisting inflamma-
MOLECULAR MECHANISMS LINKING LEPTIN AND CRP: CRP MAY ABLE TO MODULATE LEPTIN ACTION

The data reviewed above clearly demonstrate that circulating leptin and CRP levels are linked by a regulatory loop in which leptin stimulates hepatic and vascular CRP expression (Fig. 1). Interestingly, there are experimental data suggesting that a feedback-inhibitory mechanism may exist with CRP directly binding leptin and inhibiting its action (Fig. 2). Chen and colleagues reported the presence of five major serum leptin-interacting proteins (SLIPs) in human blood, isolated by leptin-affinity chromatography and identified by mass spectrometry and immunochromatography [40]. These five proteins were termed SLIP-1, SLIP-2, SLIP-3, SLIP-4 and SLIP-5, and had apparent molecular weights on silver-stained SDS-PAGE gels of 30, 42, 65, 70 and 85 kDa, respectively. All five human SLIPs had rat counterparts, as passage of rat serum through the mouse leptin affinity column yielded proteins of similar molecular weights. SLIP-1 has been identified as human CRP by mass spectrometry. SLIP-2 and SLIP-3 were treated with leptin alone, we observed a significant increase in the levels of other cytokines (soluble Tumor Necrosis Factor-α receptors, interleukin-10, monocyte chemoattractant protein-1, IL-6) were observed as compared to placebo-treated fasting subjects. While the study was not specifically designed to discriminate between the direct and indirect effects of leptin, it is worth noting that changes in proinflammatory markers levels at the end of the study significantly correlated with the leptin levels achieved, but not with leptin-induced changes in endocrine and metabolic function [46]. These results were however not replicated from other research groups [47,49]; notably when r-methHuleptin (20 mg/daily in two doses) was administered for 16-weeks to 117 obese subjects with type 2 diabetes, no significant changes in CRP levels nor in the levels of other cytokines (soluble Tumor Necrosis Factor-α receptors, interleukin-10, monocyte chemoattractant protein-1, IL-6) were observed as compared to placebo-treated matched obese diabetic subjects [49]. Taken together the results from these studies suggest that the ability of leptin to directly induce CRP in vivo is highly dependent from the leptin sensitivity state and indeed the more profound changes were observed in a state of enhanced leptin sensitivity [46], while no effect was reported in obese leptin resistant individuals [49]. It is also worth mentioning that exogenous administration of leptin may not provide an accurate picture of the actions of the adipokine in more physiological settings.

MOLECULAR MECHANISMS LINKING LEPTIN AND CRP: GENETIC VARIANTS AT THE LEPTIN RECEPTOR LOCUS ARE ASSOCIATED WITH CRP PLASMA LEVELS

An additional level to the complexity of the interactions between CRP and leptin is represented by the observation that genetic variants at the leptin receptor locus (LEPR) are independently related to circulating CRP levels. A linkage disequilibrium analysis of 71 single-nucleotide polymorphisms (SNPs) spanning the LEPR locus in a cohort of 630 healthy Caucasian individuals, revealed four haplotype blocks; of these, the fourth block was significantly associated with CRP levels (r²=0.022, P=0.049). The strongest effect was observed for the SNP rs1805096, with homozygous carriers of the major allele showing 32% higher CRP levels than carriers of the minor allele (P=0.011) [53]. The association of the LEPR locus with CRP circulating levels was confirmed by a genome-wide study in which 336,108 SNPs have been evaluated among 6,345 apparently healthy women as potential determinants of plasma CRP.


tory process (e.g., dental abscess reported in one subjects), or to mild subcutaneous inflammation at the site of injection (e.g., observed in two subjects) [44]. In a proof-of-concept study aimed to sought evidence for a proinflammatory role of leptin, pegyalted human recombinant leptin (PEG-OB 80 mg) was administered weekly by subcutaneous injections to 12 obese subjects undergoing diet-induced weight loss. The only proinflammatory molecule whose circulatory levels increased significantly upon PEG-OB administration was plasma CRP (P<0.05) [45]. In a subsequent randomized, placebo-controlled study, twenty healthy, young (18-35 yr old), normal-weight (BMI=20-26 kg/m²) female volunteers underwent a 4-d fasting during which they received r-methHu leptin at a dose sufficient to prevent the fasting-induced decline in leptin, thus maintaining physiological concentrations of the hormone. Leptin administration increased CRP levels (6.3±2.4 vs. 0.7±0.3 mg/liter; P<0.04 in leptin-treated vs placebo-treated, respectively), and stimulated other inflammatory markers, including circulating P-selectin levels and platelet aggregation, as compared to placebo-treated fasting women. While the study was not specifically designed to discriminate between the direct and indirect effects of leptin, it is worth noting that changes in proinflammatory markers levels at the end of the study significantly correlated with the leptin levels achieved, but not with leptin-induced changes in endocrine and metabolic function [46]. These results were however not replicated from other research groups [47,49]; notably when r-methHu leptin (20 mg/daily in two doses) was administered for 16-weeks to 117 obese subjects with type 2 diabetes, no significant changes in CRP levels nor in the levels of other cytokines (soluble Tumor Necrosis Factor-α receptors, interleukin-10, monocyte chemoattractant protein-1, IL-6) were observed as compared to placebo-treated matched obese diabetic subjects [49]. Taken together the results from these studies suggest that the ability of leptin to directly induce CRP in vivo is highly dependent from the leptin sensitivity state and indeed the more profound changes were observed in a state of enhanced leptin sensitivity [46], while no effect was reported in obese leptin resistant individuals [49]. It is also worth mentioning that exogenous administration of leptin may not provide an accurate picture of the actions of the adipokine in more physiological settings.

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concentration. Overall, seven loci associated with plasma CRP at levels achieving genome-wide statistical significance have been identified, including loci in or near the CRP gene itself, and the leptin receptor protein gene. Notably, virtually identical results were observed when the analysis was restricted to the study participants with CRP levels < 10 mg/l, characteristic of chronic inflammation [54].

CONCLUSIONS

The data reviewed here demonstrate that a complex interplay links plasma leptin and plasma CRP levels. Clinical data underline the importance of both markers in estimating CVD risk and strongly suggest that an additional value will be attained by assessing them in tandem, especially in clinical states, such as obesity, where chronically elevated CRP levels and leptin resistance coexist [27-38]. Molecular studies indicate that leptin is able to modulate CRP expression levels, both indirectly, throughout its action on other proinflammatory molecules, such as IL-6, and directly promoting its hepatic and vascular production [40,42,43]. In turn, CRP may be able to regulate circulatory leptin bioavailability, as it has been demonstrated that in extracellular settings the two molecules coprecipitate [40, 52] and that this interaction impairs leptin ability to bind its receptor and activate intracellular signaling [52]. If confirmed by future in vivo studies in humans, these findings may highlight a potential link between conditions, such as leptin resistance and endothelial dysfunction, that may be amenable of pharmacological treatment targeted to the disruption of leptin-CRP interaction. Notably, as reported above, leptin induced-vascular NO production is impaired in obesity/metabolic syndrome, conditions characterized by hyperleptinemia, higher plasma CRP levels and leptin resistance [6-8]. Therapies aimed to improve the beneficial effects of leptin by modulating its intracellular signal transduction might thus be helpful in prevention and treatment of leptin resistance-related cardiovascular pathologies. On the other hand, CRP, in addition to being a marker of inflammation, has been shown to exert proatherogenic effects on endothelial and vascular smooth muscle cells [19-21]. Pharmacological agents able to reduce CRP production may therefore not only be useful to prevent the direct negative effects of CRP, but also to increase leptin bioavailability. Finally, several studies have demonstrated that weight loss and physical aerobic activity are associated with a decrease in CRP and leptin levels as well as with improved endothelial function and decreased CVD risk [55-58]. These findings, in addition to confirming the link between leptin, CRP levels and CVD, point to weight loss and physical activity as therapeutic approaches able to break the vicious circle feeding CVD pathogenesis.

CONFLICT OF INTEREST

The authors confirm that this article content has no conflicts of interest.

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