

EXTENDED REPORT

Markers of inflammation are negatively correlated with serum leptin in rheumatoid arthritis

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Background: Leptin regulates food intake and modulates immunity and inflammation. A positive feedback mechanism has been described between tumour necrosis factor (TNF) and leptin, and it has been suggested that leptin potentiates inflammation in patients with rheumatoid arthritis (RA).

Objective: To assess whether inflammation correlates with leptin concentrations in patients with RA, and whether anti-TNF treatment modulates leptin concentrations in these patients.

Methods: Leptin, IL6 and CRP were measured (at baseline and after 2 weeks of treatment) in the blood of 31 patients with RA starting either anti-TNF treatment or placebo, and in 18 healthy controls.

Results: In patients with RA, plasma leptin concentrations at baseline correlated inversely with the degree of inflammation as assessed by C reactive protein (CRP; $r_s^2=0.21$, $p<0.01$) or interleukin (IL) 6 concentrations ($r_s^2=0.22$, $p<0.008$). Mean (SD) leptin concentrations did not differ between patients with RA and controls (6.0 (4.6) v 4.2 (2.8) ng/ml in men; 15.1 (7.9) v 13.4 (5.2) ng/ml in women). Short course anti-TNF treatment for 2 weeks did not modify leptin concentrations, despite significant reduction of CRP and IL6.

Conclusion: A significant inverse correlation between inflammation and leptin concentrations was found in patients with active RA, although plasma leptin concentrations did not significantly differ from those in healthy controls. This suggests that active chronic inflammation may lower plasma leptin concentrations. Two weeks' treatment with anti-TNF did not change plasma leptin concentrations and longer treatment may be needed to see an effect on leptin.

Leptin was initially described as a hormone that regulates food intake and energy balance.¹ Later, it became apparent that leptin has an important role in regulating neuroendocrine and immune functions. Leptin and its receptors (OB-R) share structural and functional similarities with cytokines of the interleukin (IL) 6 family and their receptors.² During acute inflammation, proinflammatory cytokines increase circulating leptin concentrations,³ and leptin, in turn, potentiates cytokine release from monocytes/macrophages.⁴ In addition, leptin stimulates T cell mediated immunity and induces the proliferation and differentiation of haematopoietic cells.³ Regulation of immune functions in humans is strongly sustained by the increased incidence of severe infections in subjects with genetic leptin deficiency⁵ and by the deficiencies of the immune system during starvation and malnutrition, when concentrations of leptin are low.³

Rheumatoid arthritis (RA) is a chronic inflammatory condition characterised by polyarthritis and high concentrations of proinflammatory cytokines such as tumour necrosis factor α (TNF α), IL1 β , IL6, IL8, and interferon γ , especially in the synovial fluid but also in the circulation. A dual effect of inflammation on leptin production has been suggested. On the one hand, a positive feedback between leptin and proinflammatory cytokines has been reported,⁴ and immunised leptin deficient mice (ob/ob) were shown to develop less severe arthritis than control mice.⁶ Recently, the relation between leptin and arthritis was further supported by studies showing that human chondrocytes express the leptin receptor OB-Rb and, when acting together with interferon γ , leptin stimulated nitric oxide production in the joint cavity.⁷ This suggests that leptin may be directly implicated in the pathogenesis of RA. On the other hand, results of studies assessing leptin concentrations in patients with RA have been controversial.^{8–12} Additionally, it has been suggested that

chronic inflammation down modulates leptin production, which in turn may lead to an impaired antimicrobial defence.¹³

Our study aimed at investigating circulating leptin concentrations in a group of patients with RA and at assessing whether leptin concentrations correlate with systemic inflammation. In addition, we were interested to determine whether anti-TNF treatment modulates plasma leptin concentrations, as TNF has been shown to stimulate leptin production directly.

PATIENTS AND METHODS

Patients and controls

We analysed samples from 31 patients with active RA (mean age 61, M:F = 11:20) included in a phase I, double blind, placebo controlled clinical study of monotherapy with the humanised anti-TNF antibody adalimumab (Humira, Abbott Laboratories) monotherapy at our centre. Patients fulfilled the 1987 American College of Rheumatology criteria for RA, had active disease as defined by a Disease Activity Score >3.2 at baseline, and underwent a washout period for disease modifying antirheumatic drugs (DMARDs) of at least 3 weeks before the start of the study. Stable dosages of non-steroidal anti-inflammatory drugs and prednisone (<10 mg/day) were allowed during the study. Eighteen healthy controls (mean age 38.4, M:F = 9:9) were also included in this study. Because of the differences in leptin concentrations between men and women and because the ratio of men to women differed in the patient (M:F = 1:1.8) and control (M:F = 1:1) groups, we divided each group according to sex before comparing them. Body mass index

Abbreviations: BMI, body mass index; CRP, C reactive protein; DMARDs, disease modifying antirheumatic drugs; IL, interleukin; RA, rheumatoid arthritis; TNF α , tumour necrosis factor α

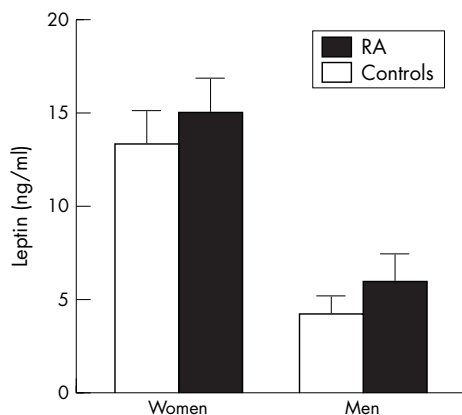


Figure 1 Circulating leptin concentration in patients with RA (n=31) and controls (n=18). No significant differences were found for men or for women.

(BMI), calculated as weight/height² (kg/m²), was used to obtain a better comparison of leptin concentrations between groups.

To investigate the effect of TNF blockade on circulating leptin concentrations, we compared the samples from 23 patients with RA treated with anti-TNF and eight patients with RA receiving placebo at baseline and after 2 weeks of treatment.

Leptin, IL6, and C reactive protein (CRP)

Fasting blood samples were collected in Vacutainer tubes (Becton & Dickinson, Rutherford, NJ) containing K3-EDTA (1 mg/ml), centrifuged at 3600 rpm for 8 minutes at 4°C, supplemented with saccharose as a cryoprotectant (final concentration 6 mg/ml), and frozen at -80°C until assay. Leptin and IL6 were determined by a commercial enzyme linked immunosorbent assay (ELISA; Biosource, Etten-Leur, The Netherlands; detection limits 32 pg/ml and 1.5 pg/ml, respectively), according to the manufacturer's instructions. CRP was measured by immunoturbidometry with the Hitachi 747 analyser using reagents obtained from Roche (Nos 1776371 and 1776428) and the calibrator BCD1. The sensitivity level was 1 mg/l and the coefficient of variation <2%.

Statistical analysis

Groups were compared using the Mann-Whitney non-parametric U test and Student's *t* test for parametric data. Paired group comparisons during treatment were made with Wilcoxon's signed rank test. Correlation was calculated using Spearman's non-parametric test. The relation between

plasma leptin concentration and CRP/IL6 was analysed by linear regression. Results are expressed as means (SD). Significance was set at $p < 0.05$.

RESULTS

Circulating leptin concentrations do not differ between patients with RA and healthy controls

To compare leptin concentrations in our patients with RA and controls, we examine men and women separately. We found no significant differences in circulating leptin concentrations between patients with RA and controls either in men (6.0 (4.6) ng/ml *v* 4.2 (2.8) ng/ml) or in women (15.1 (7.9) ng/ml *v* 13.4 (5.2) ng/ml) (fig 1). Similar results were obtained when leptin concentrations were adjusted for BMI: 0.2 (0.1) ng/ml *v* 0.2 (0.1) ng/ml in men and 0.7 (0.3) ng/ml *v* 0.6 (0.2) ng/ml in women. However, the BMI was significantly higher in patients with RA than in controls both in men (25.5 (2.9) kg/m² *v* 23.1 (2.4) kg/m², $p < 0.032$) *v* women (26.2 (4.9) kg/m² and 22.4 (2.6) kg/m²; $p < 0.041$).

Inflammation is negatively correlated with plasma leptin concentration

Leptin concentrations in plasma are mainly regulated by body fat mass, and correlate with body fat mass and with BMI in healthy subjects. In our study circulating leptin concentrations correlated with BMI in healthy men ($r_s = 0.88$, $p = 0.003$) and women ($r_s = 0.70$, $p = 0.043$). Interestingly, no such correlation was seen in men ($r_s = 0.17$, $p = \text{NS}$) or women ($r_s = 0.15$, $p = \text{NS}$) with RA before starting anti-TNF treatment.

Inflammation, in addition to body mass fat, also helps to regulate plasma leptin concentrations. In the group with RA, the markers of inflammation CRP and IL6 were found to be higher than normal. To analyse the relation between plasma leptin concentration and CRP/IL6, we used a linear regression test. In patients with RA, before starting anti-TNF treatment, plasma concentrations of leptin inversely correlated with CRP (fig 2A) and IL6 levels (fig 2B). The same results were obtained when leptin concentrations were adjusted for BMI: $r_s^2 = 0.22$, $p < 0.008$ when correlated with CRP concentrations, and $r_s^2 = 0.20$, $p < 0.01$ when correlated with IL6 concentrations at baseline.

The duration of disease in our RA group varied between 3 and 26 years, with an average of 11 years, and was not associated with serum leptin concentrations. Additionally, we found no relation between the previous use of DMARDs and serum leptin concentrations at baseline.

Effects of anti-TNF treatment on inflammatory markers and plasma leptin concentrations

As we reported previously,¹⁴ the levels of CRP and IL6 decreased significantly within 2 weeks of anti-TNF

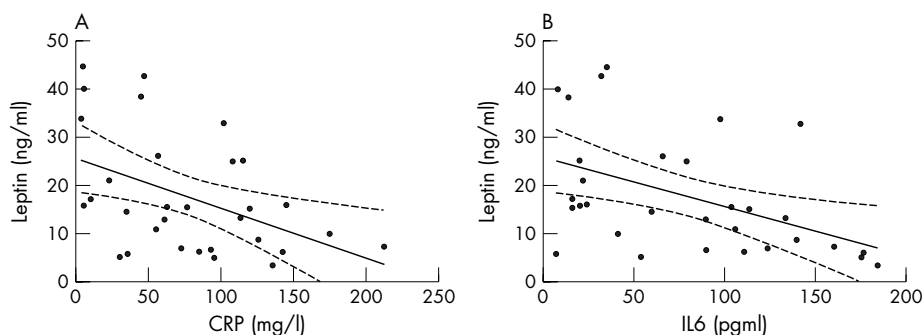


Figure 2 Circulating leptin concentrations in patients with RA (n=31) correlate negatively with (A) CRP ($r_s^2 = 0.21$, $p < 0.01$) and (B) IL6 ($r_s^2 = 0.22$, $p < 0.008$). A linear regression test was used.

Table 1 Evaluation of the inflammatory status, as assessed by levels of CRP and IL6, and plasma leptin concentrations in patients with RA after 2 weeks' anti-TNF treatment (n = 23) or placebo (n = 8)

	Week 0	Week 2	p Value
<i>Leptin (ng/ml)</i>			
Men (n = 10)	6.2 (5.0)	5.7 (3.5)	NS
Women (n = 13)	15.4 (8.7)	16.7 (9.9)	NS
<i>C reactive protein (mg/ml)</i>			
Anti-TNF	86.1 (54.4)	35.4 (35.6)	<0.0001
Placebo	53.7 (49.2)	53.9 (50.0)	NS
<i>Interleukin 6 (pg/ml)</i>			
Anti-TNF	88.3 (60.5)	42.3 (40.7)	<0.001
Placebo	60.0 (59.6)	60.8 (60.2)	NS

Results are given as mean (SD).

administration, whereas no changes were observed with placebo (table 1).

After 2 weeks of anti-TNF treatment, plasma leptin concentrations in patients with RA were similar to those at baseline both in men and women (table 1). Moreover, no significant differences were seen in the placebo treated group.

DISCUSSION

Inflammatory mediators, such as the cytokines TNF α and IL1 β , decrease energy intake and may lead to the wasting described in patients with RA. Wasting, in turn, affects the inflammatory response and may suppress cellular immunity. In this complex relationship, leptin is a possible mediator. In this study we show that in patients with RA, both circulating leptin concentrations and leptin adjusted for the BMI, inversely correlated with the inflammatory status of the patients, as assessed by the inflammatory markers CRP and IL6. These results are supported by the observations that long term in vitro stimulation of adipose tissue by TNF α or IL1 β inhibits leptin and leptin mRNA production.¹⁵ Similarly, in patients with tuberculosis, another chronic inflammatory condition, inflammation correlates negatively with leptin concentration.¹³ In patients with RA, plasma leptin concentrations did not correlate with BMI, suggesting that regulation of leptinaemia in RA is complex, and that weight is not the only major regulator. These facts led us to propose the hypothesis that in RA chronic inflammation, probably through proinflammatory cytokines (for example, TNF, IL1, IL6), is an important determinant of plasma leptin concentration and has an inhibitory effect on leptin production.

In addition, we report that plasma leptin concentrations in patients with RA do not differ from those found in healthy controls. This is in line with two earlier studies.^{8,9} In contrast, Bokarewa *et al* found higher plasma leptin concentrations in a group of patients with RA.¹¹ Theoretically, one would expect increased leptin concentration owing to the proinflammatory status of RA and to the stimulatory activity of TNF α and IL1 β on leptin release.^{3,15} Similarly, patients with sepsis and those who had had major surgery, two situations also characterised by increased TNF α and IL1 β concentrations, had raised serum leptin concentrations.¹⁶ However, as shown above, chronic inflammation in patients with RA had inhibitory effects on leptin concentrations in the blood, in contrast with the acute inflammation of sepsis and surgery. Recently, Harle *et al* found that serum leptin concentrations were almost three times lower in a group of women with RA than in a group of healthy women.¹² In addition, the body compartment in which leptin is measured may be of importance. Although blood concentrations of leptin did not differ

significantly between patients with RA and controls, concentrations in the synovial fluid may be of importance.^{7,11}

The lack of difference between plasma leptin concentrations in patients with RA and healthy controls may seem in contrast with the inverse correlation of leptin and inflammation in these patients, which suggests that there will be lower leptin concentration in patients with RA. The cause of this discrepancy is probably due to a combination of factors: a significant percentage of the patients with RA did not have very high inflammatory parameters at the time of investigation; the BMI of the patients with RA in our group was slightly higher than that of the control volunteers; and some of the inhibitory effects of chronic inflammation might have been counterbalanced by potential stimulatory actions of acute inflammatory reactions during exacerbations of RA.

We also evaluated a possible correlation of the duration of the disease with plasma leptin concentrations, but no direct relation between these two measures was found. These results are in line with those of Anders *et al*,⁸ whereas Bokarewa *et al* showed a gradual increase of leptin concentrations with the duration of RA.¹¹

To explain our results better we evaluated the influence of previous treatment with DMARDs on serum leptin concentration in our RA group. We could not establish a relation between leptin concentrations at baseline and this treatment, no matter which type of drug or what dosage was used. Bokarewa *et al* found higher leptin concentrations in a group of patients with RA treated with methotrexate than in a group receiving other DMARDs, but at the same time, these concentrations were similar to those found in a group of patients who were not treated with any DMARDs. In the same study no difference in serum leptin concentrations was found between patients with RA treated and not treated with glucocorticoids.¹¹ Sulfasalazine has also been shown to have no influence on leptin release from adipose tissue and skeletal muscle.¹⁷ The above mentioned studies suggest that no one specific DMARD influences serum leptin concentrations. Moreover, a washout period for DMARDs was performed on every patient included in our study 3 weeks before entry—that is, before the time at which blood was collected for leptin determination.

Leptin is known to stimulate T cell mediated immunity. In the case of septic shock, mortality is associated with decreased plasma leptin levels,¹⁸ while genetic leptin deficiencies increase the severity of infections in humans.⁵ In addition, severe infections have been reported to occur more often in patients with RA than in the general population,¹⁹ especially in patients receiving anti-TNF drugs.²⁰ These data suggest that suppression of leptin concentration by chronic inflammation may contribute to the susceptibility of patients with RA to infections.

An additional aim of our study was to investigate whether a short course of anti-TNF treatment can influence leptin concentrations. To date, as far as we know, no study has investigated the effect of in vivo TNF α blockade upon circulating leptin levels. We found that a 2 week course of anti-TNF treatment did not change plasma leptin concentrations, despite decreasing the acute phase reactants. Therefore, a short course of treatment with anti-TNF does not modulate leptin concentrations in patients with RA, and studies investigating the effect of long term treatment on leptin concentration are warranted.

In conclusion, our study shows that circulating leptin concentrations are inversely correlated with the inflammatory status in patients with RA. We suggest that in RA, chronic inflammation down regulates leptin production, which may indirectly contribute to the susceptibility to infections seen in these patients. The precise role of leptin in RA remains uncertain but, possibly, local actions, through

synovial leptin, play a part in the pathogenesis of the disease, while decreased circulating leptin contributes to impaired host defence to infections. A short course of anti-TNF treatment does not modify plasma leptin concentrations and therefore longer follow up studies are needed to assess this issue further.

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