Anti-TNF-α therapy does not modulate leptin in patients with severe rheumatoid arthritis

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Abstract

Objectives

The adipocytokine leptin regulates weight centrally and participates in the regulation of the immune and inflammatory responses. Chronic systemic inflammation is of major importance in the development of atherosclerosis in rheumatoid arthritis (RA). In the present study we investigated whether inflammation, obesity or both of these characteristics are potential determinants of circulating leptin concentrations in a group of RA patients on periodical treatment with the TNF-α-blocker-infliximab due to severe disease. We also assessed whether the infusion of infliximab may alter circulating leptin concentrations in patients with severe RA.

Methods

We investigated 33 patients with RA on periodical treatment with infliximab. Serum leptin levels were determined immediately prior to and after infliximab infusion.

Results

There was a positive correlation between body mass index of RA patients and baseline serum level of leptin (rho=0.665, p<0.001). Apart from a significant correlation with VCAM-1 (rho=0.349, p=0.04), no significant correlations between baseline leptin levels and the age at the time of the study or at the onset of the disease, disease duration, ESR and CRP levels, DAS28, lipids, insulin sensitivity, adhesion molecules, resistin, adiponectin, ghrelin or the cumulative prednisone dose at the time of the study were found. Leptin levels did not change upon infliximab infusion (p=0.48).

Conclusions

In RA patients on TNF-α blocker treatment, circulating leptin levels are unrelated to disease activity but constitute a manifestation of adiposity. The beneficial effect of anti-TNF-α therapy on cardiovascular mortality in RA does not seem to be mediated by reduction in serum levels of leptin.

Key words

Rheumatoid arthritis, inflammation, circulating leptin, anti-TNF-α antibody-infliximab, cardiovascular risk.

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List of abbreviations:

CV:	cardiovascular
BMI:	body mass index
CRP:	C-reactive protein
DAS28:	Disease Activity Score-28
DMARD:	disease modifying anti-rheumatic drug
ESR:	erythrocyte sedimentation rate
HDL:	high density lipoprotein
HOMA-IR:	homeostasis model assessment of insulin resistance
ICAM-1:	intercellular cell adhesion molecule-1
ICAM-3:	intercellular cell adhesion molecule-3
IL:	Interleukin
LDL:	low density lipoprotein
RA:	rheumatoid arthritis; s = soluble
TNF-α:	tumor necrosis factor-α
VAS:	visual analogue scale
VCAM-1:	vascular cell adhesion molecule-1

Competing interests: none declared.

Introduction

Rheumatoid arthritis (RA) is a chronic autoimmune disease associated with accelerated atherosclerosis and increased risk of cardiovascular (CV) events (1, 2). Besides traditional CV risk factors (3, 4) and genetic predisposition (5, 6), chronic systemic inflammation is of major importance in the progression of atherosclerosis and the increased incidence of CV events observed in these patients (6, 7).

Leptin is an adipocytokine that plays an important role in the regulation of body weight by inhibiting food intake and stimulating energy expenditure (8). Leptin is also a proinflammatory adipocyte-derived factor that operates in the cytokine network by linking immune and inflammatory processes to the neuroendocrine system (8, 9). Leptin regulates and participates both in immune homeostasis and in inflammatory processes. In this regard, leptin acts as a modulator of T-cell activity and plays a key role in some autoimmune inflammatory diseases such as type 1 diabetes, bowel inflammation and RA (8, 9). Furthermore, this adipokine is produced by stimulation of inflammatory cytokines such as tumor necrosis factor (TNF)-α and interleukin (IL)-1. Importantly, leptin exerts many potential atherogenic effects and high leptin concentrations predict incident CV disease in non-RA subjects (10).

Chronic increase of proinflammatory cytokines such as TNF-α, IL-1β, and IL-6 causes deleterious effects including a proatherogenic lipid profile, insulin resistance and endothelial dysfunction in RA (7). TNF-α blockers are highly effective in the treatment of RA and they have been found to reduce CV mortality more than traditional disease modifying rheumatic drugs (DMARD) (11). In this regard, marked improvement of endothelial function following anti-TNF-α blockade using the chimeric anti-TNF-α monoclonal antibody-infliximab was observed in RA with severe disease on periodical treatment with this drug. (12). Moreover, in keeping with other investigators (13), following infliximab infusion, RA patients with severe disease experienced a rapid improvement in insulin sensitivity (14) and also a reduction in the levels of adhesion molecules associated with atherogenesis (15).

In assessing a series of RA patients with severe disease, refractory to conventional DMARD therapy, on periodical treatment with the TNF-α blocker- infliximab, we recently studied whether inflammation, obesity or both of these characteristics were potential determinants of circulating concentrations of the adipokine adiponectin, and whether low adiponectin concentrations cluster with metabolic syndrome features (16). We found, in this cohort, that high-grade inflammation was independently and negatively correlated with circulating adiponectin concentrations whereas low adiponectin levels clustered with metabolic syndrome features that reportedly contribute to atherogenesis in RA (16) but no changes on adiponectin levels were found upon infliximab administration (16). However, in the same cohort of RA patients, we found a significant positive correlation between laboratory markers of inflammation, particularly C-reactive protein (CRP), with the serum levels of the adipocyte-derived mediator resistin (17). Moreover, anti-TNF-α infliximab therapy induced a rapid and significant reduction of serum resistin levels in RA patients with severe disease (17).

In view of the above-mentioned reported findings, in the present study, we investigated whether inflammation, obesity or both of these characteristics are potential determinants of circulating leptin concentrations in a group of RA patients on periodical treatment with the TNF-α-blocker infliximab due to severe disease (15-17). We also assessed whether infliximab infusion might alter circulating leptin concentrations in patients with severe RA.

Patients and methods

Patients

We investigated 33 consecutive patients that met the 1987 American College of Rheumatology criteria for RA (18) and that were recruited from Hospital Xeral-Calde, Lugo, Northwest Spain. They formed part of an ongoing study on CV disease in RA (12, 14-17, 19).

Each of the RA patients had been

switched from traditional DMARD to anti-TNF-α infliximab treatment because of severe and active disease (Disease Activity Score-28 [DAS28] >5.1) (15, 20). In all patients, treatment with a DMARD had been initiated when a diagnosis of RA was made. Prior to anti-TNF-α therapy, patients were required to have been treated with at least two DMARDs including chloroquine, sulphasalazine, gold, methotrexate (at least 15 mg/week), leflunomide, and cyclosporine A (3 mg/kg/day). Infliximab therapy (initial dose of 3 mg/kg) was administered intravenously at 0, 2, 6 weeks and subsequently every 8 weeks. However, in some patients, because of disease severity, the dose was increased to 5 mg/kg and, if deemed necessary, the interval between infliximab infusions was shortened to 6 weeks.

All patients had received treatment with both non-steroidal antiinflammatory agents and low doses of prednisone (generally 5 mg bid) immediately after disease diagnosis. At the time of the study, each patient was on infliximab 3 or 5 mg/kg given at 6 or 8 weekly intervals (range of treatment duration: 1-4.5 years), oral methotrexate 15-25 mg weekly with or without chloroquine 250 mg daily, prednisone 2.5-7.5 mg daily and a non-steroidal antiinflammatory agent (naproxen 500-1000 mg or diclofenac 50-100 mg daily). The blood pressure was below 140/90 mmHg in each patient at the time of the study. However, 7 were taking antihypertensive agents (enalapril [n=3]; losartan [n=3]; enalapril and hydrochlorothiazide [n=1]). Four patients were using a statin (simvastatin 20-40 mg daily). Patients with diabetes were excluded. For ethical reasons, patients included in the present study were not randomized to a placebo group. The same procedure has been found acceptable and followed in a recent study on the effect of infliximab therapy on lipid profiles in patients with RA (21). The local institutional committee approved anti-TNF-α therapy and each patient gave informed consent to participate in the study. Neither this study nor previous studies on RA patients receiving periodical treatment with infliximab (12, 14-17, 22) were supported by any pharmaceutical drug company.

Study protocol

As previously reported (14,15), in each patient a DAS28 (20) was recorded by the same rheumatologist (MAG-G) prior to infliximab infusion (the same day). In all cases, the drug was given at 8 a.m. as an intravenous infusion in a saline solution over 120 minutes. None of the patients received any nutrient before and during infusion.

All measurements were made in the fasting state. Blood samples were taken at 0800 hours for determination of the erythrocyte sedimentation rate-ESR, CRP, lipids, plasma glucose and serum insulin levels that were determined as previously reported (14-17).

Serum leptin (human leptin was measured by immunoradiometric assay (RIA) kit [Linco Research, St. Charles, MO, USA]; assay sensitivity was 0.5 ng/ml and the intra- and interassay coefficients of variation were <6% and <7% respectively), immediately prior to an infliximab infusion.

Insulin resistance was estimated by the homeostasis model assessment of insulin resistance (HOMA-IR) using the formula = (insulin (μU/ml) x glucose (mmol/l)÷22.5[7]. Also, as previously reported (15), soluble (s) circulating levels of adhesion molecules, intercellular cell adhesion molecule-1 (ICAM-1), ICAM-3, vascular cell adhesion molecule-1 (VCAM-1), E-selectin and P-selectin, and serum adiponectin (16), resistin (17) and ghrelin (22) were measured prior to infliximab infusion.

Subsequently, final blood sampling was performed for determination of leptin, adiponectin, resistin, ghrelin and adhesion molecules concentrations immediately after infliximab that was administered over 120 minutes.

Statistical analyses

Results were expressed as mean ± standard deviation (SD), median and interquartile (IQ) range, or number (n) (%). The associations between baseline characteristics and serum leptin concentrations (expressed as mean ± SD, median and IQ range) were assessed by estimating the Spearman correlation coefficient (rho) for continuous variables, as leptin concentrations were not normally distributed. Associations between leptin concentrations and gender, hypertension and treatment with chloroquine and statins were assessed by the Mann-Whitney U-test. Differences in basal leptin concentrations by disease severity (DAS28>5.1 *vs.* DAS≤5.1) and by quartiles of CRP concentration were analyzed by Kruskal-Wallis test. The changes in serum leptin concentrations upon infliximab therapy (just prior to infusion at time 0 and immediately after the end of infliximab infusion at time 120 minutes) were evaluated using the paired Student's *t*-test. Statistical significance was accepted at $p< 0.05$.

Results

Descriptive data

The baseline-recorded variables in this series of 33 RA patients on periodical treatment with infliximab are shown in Table I. Despite clinical improvement, as reflected by a reduction in the DAS28 score compared to that found prior to the onset of anti-TNF-α therapy, all except one patient still had active disease (DAS28>2.6) (23).

Correlations between the basal recorded characteristics and serum leptin concentrations

Although leptin concentrations (ng/ml) were higher in women with RA (18.0±16.3) than in men (10.5±12.2), this difference was not statistically significant (p=0.09). Also, no statistical differences were found between leptin concentrations in patients treated with chloroquine (14.8±15.7) versus in those not on chloroquine (24.1±13.3) (p=0.21) or in RA patients treated with a statin (26.2±21.8) versus those not on statins (14.4±13.9) (p=0.16); however, these results should be carefully interpreted as only 4 patients were on statins and 5 were not on chloroquine.

No significant correlations between leptin concentrations obtained before infliximab administration and the age at the time of the study or at the onset of the disease and disease duration were found. Although there was a weak negative correlation between VAS patient's disease activity and leptin concentrations (rho= -0.349, p=0.04), no significant correlations between tender and

Table I. Baseline characteristics in 33 rheumatoid arthritis patients on treatment with anti-TNF-α therapy. Results are expressed as n (%), mean± standard deviation (SD); median (interquartile range-IQ).

	n (%)	Mean± SD	median (IQ)
Age, years			
At disease onset		43.3 ± 12.0	42 (37-57)
At the time of the study		55.3 ± 12.8	55 (46-65)
Women	25 (76)	---	---
Disease duration, years		12.3 ± 7.5	11 (5-16)
Time from the onset of RA to the beginning of infliximab therapy, years		10.0 ± 7.3	9 (4-15)
Rheumatoid factor positive	30 (91)	---	---
Disease activity			
DAS28		4.4 ± 1.1	4.4 (3.6-5.1)
Swollen joint count, n		4.8 ± 4.0	3 (2-7)
Tender joint count, n		4.1 ± 3.7	3 (1-6)
VAS patient disease activity		41.2 ± 17.0	40 (30-50)
CRP at the time of the study, mg/l		14.2 ± 16.0	5.5 (4.0-23.4)
Mean CRP from disease diagnosis, mg/l		20.9 ± 12.6	16 (12-28)
ESR at the time of the study, mm/hr		30.2 ± 19.4	28 (16-39)
Mean ESR from disease diagnosis, mm/hr		36.6 ± 20.8	31 (23-45)
Platelet count at the time of the study, $x10^9$/l		289.9 ± 81.6	270 (245-327)
Cumulative prednisone dose, gr		28.72 ± 18.86	27.45 (10.60-48.25)
Years of treatment with infliximab		2.5 ± 1.2	2.0 (1.5-3.5)
Metabolic syndrome features			
Body mass index, kg/m^2		25.4 ± 4.4	24.0 (22.6-28.9)
Hypertension	7 (21)	---	---
Systolic blood pressure, mmHg		120.2 ± 10.9	120 (115-130)
Diastolic blood pressure, mmHg		73.3 ± 7.1	75 (70-80)
Glucose, mmol/l		4.85 ± 0.78	4.88 (4.27-5.28)
Insulin, pmol/l		107.6 ± 69.5	84.7 (59.7-131.2)
HOMA-IR, μU.mmol/ml.l		3.4 ± 2.3	2.9 (1.9-4.2)
Total cholesterol, mmol/l		4.97 ± 0.80	5.12 (4.42-5.49)
HDL cholesterol, mmol/l		1.64 ± 0.31	1.63 (1.45-1.86)
LDL cholesterol, mmol/l		2.70 ± 0.51	2.74 (2.33-3.13)
Triglycerides, mmol/l		1.22 ± 0.50	1.15 (0.98-1.56)
Adiponectin, ng/ml		25790 ± 28122	15705 (11240-26180)
Resistin, ng/ml		21.9 ± 9.9	18.8 (15.0-26.8)
Leptin, ng/ml		16.2 ± 15.5	10.9 (5.3-19.4)
sICAM-1, ng/ml		349.8 ± 103.2	345.0 (288.4-395.6)
sICAM-3, ng/ml		58.4 ± 15.1	53.9 (48.6-64.2)
sVCAM-1, ng/ml		1098 ± 370	1013 (882-1254)
sE-selectin, ng/ml		53.0 ± 27.9	42.4 (33.5-67.4)
sP-selectin, ng/ml		291 ± 356	221 (141-316)
ghrelin, pg/ml		896.1 ± 314.8	861.2 (700.5-879.9)

DAS: disease activity score; VAS: visual analogue scale; BMI: body mass index; HOMA-IR: homeostasis model assessment of insulin resistance; HDL: high-density lipoprotein; LDL: low-density lipoprotein.

swollen joints, DAS28, the mean ESR and CRP from disease diagnosis and the ESR, CRP and platelet count at the time of the study or the cumulative prednisone dose and baseline leptin concentrations were observed (Table II).

Relationships of leptin concentrations with metabolic syndrome features

There was a positive correlation between body mass index (BMI) of RA patients and baseline serum level of leptin (rho=0.665, $p<0.001$) (Fig. 1). Leptin concentrations did not show a significant correlation with the HOMA-IR (rho=0.219, $p=0.27$) and with basal insulin (rho= 0.161, $p=0.38$) (Table II). Leptin concentrations (ng/ml) did not differ in patients with hypertension (22.1±13.0) versus in patients without hypertension (14.6±16.0) ($p=0.10$). Moreover, leptin concentrations were not significantly correlated with total cholesterol, HDL and LDL cholesterol, triglycerides and plasma glucose levels (Table II).

Relationships of baseline leptin concentrations with other adipokines, ghrelin or with adhesion molecules

As shown in Table II, there was no significant correlation between basal leptin and adiponectin, resistin and ghrelin concentrations. Likewise, apart from VCAM-1 (rho=0.349; $p=0.04$), no significant correlations were found between serum leptin levels and concentration of adhesion molecules (ICAM-1, ICAM-3, E-selectin and P-selectin) (Table II).

Differences in baseline leptin concentrations according to disease activity

To establish if leptin concentrations in patients with severe disease had greater concentrations of serum leptin than those with mild or moderate RA disease activity, leptin concentrations in 8 subjects with severe and active disease at the time of this study as reflected by a DAS28>5.1 were compared with those in the remaining 25 RA patients that had DAS28 ≤5.1 before infliximab infusion. Patients with a DAS28>5.1 had lower concentrations (12.1±10.6 ng/ml) than those with DAS28 ≤5.1 (17.1±16.6 ng/ml) but this difference was not significant ($p=0.52$). Moreover, RA patients were split in quartiles of CRP levels prior to infliximab infusion. Again, leptin concentrations did not differ significantly amongst groups when patients were stratified by basal CRP concentrations (1^{st} quartile: 10.6±7.8 ng/ml; 2^{nd} quartile: 27.6±20.5; 3^{rd} quartile: 21.8±20.8; 4^{th} quartile: 9.3±5.8; $p=0.23$).

Differences in baseline leptin concentrations according to patient subgroups

Since there were large differences in leptin concentration between patients taking chloroquine or not taking this drug, we again repeated the analysis assessing only the subgroup of RA patients who were not on chloroquine treatment. The results substantially coincided with those shown in Table II when we assessed the whole series of

Table II. Association between baseline patient characteristics and serum leptin levels in 33 patients with rheumatoid arthritis.

Patient characteristics	Leptin (ng/ml) rho
Age, years	
At disease onset	0.017
At the time of the study	-0.098
Disease duration, years	-0.160
Time from the onset of RA to the beginning of infliximab therapy, years	-0.135
Disease activity	
DAS28	-0.152
Swollen joints	-0.211
Tender joints	-0.020
VAS patient disease activity	-0.349*
CRP protein at the time of the study, mg/l	-0.061
Mean CRP from disease diagnosis, mg/l	-0.249
ESR at the time of the study, mm/hr	-0.025
Mean ESR from disease diagnosis, mm/hr	-0.028
Platelet at the time of the study, hundred/mm^3	-0.223
Metabolic syndrome	
BMI, kg/m^2	0.665**
Basal glucose, mg/dl	-0.020
Basal Insulin, μU/ml	0.161
Basal HOMA-IR, μU.mmol/ml.l	0.219
Triglycerides, mg/dl	0.141
Total cholesterol, mg/dl	0.097
HDL cholesterol, mg/dl	0.138
LDL cholesterol mg/dl	-0.034
Systolic blood pressure, mmHg	-0.070
Diastolic blood pressure, mmHg	0.052
Cumulative prednisone dosage, gr	0.061
Adiponectin, ng/ml	0.132
Resistin, ng/ml	0.052
sICAM-1, ng/ml	0.107
sICAM-3, ng/ml	-0.061
sVCAM-1, ng/ml	0.349***
sE-selectin, ng/ml	0.065
sP-selectin, ng/ml	-0.156
Ghrelin	0.042

DAS: disease activity score; VAS: visual analogue scale; BMI: body mass index; HOMA-IR: homeostasis model assessment of insulin resistance; HDL: high-density lipoprotein; LDL: low-density lipoprotein.
*p=0.04, **p<0.001, ***p=0.04.

33 patients. In this regard, VAS disease activity remained negatively correlated with leptin concentrations (rho= -0.430, p=0.02), while BMI continued being positively correlated (rho=0.544, p=0.003). However, the correlation between VCAM-1 and leptin did not remain significant (rho=0.325, p=0.09). Additional analyses of patient subgroups according to gender, with or without hypertension and with or without statin therapy did not influence the main correlations with leptin shown in Table II (data not shown).

Changes in leptin concentrations upon infliximab therapy

Leptin concentrations (ng/ml) did not change upon administration of an infliximab infusion (before: mean ± SD: 16.2±15.5; median: 10.9; IQ range: 5.3-19.4; after 15.9±15.6; 10.2; 6.0-19.6; p=0.48) and baseline leptin concentrations were strongly correlated with leptin concentrations after an infliximab infusion (rho=0.986, p<0.001).

The correlations of post infliximab circulating leptin concentrations with the baseline recorded characteristics (Table II) did not differ from the correlations of baseline circulating leptin concentrations with the baseline recorded characteristics (as described above) in both univariate and multivariable analysis (data not shown). Also, apart from ICAM-1 (rho=0.403, p=0.02), changes in serum leptin concentrations after 120 minutes of the infliximab infusion did not show a significant correlation with changes in adiponectin, resistin, ghrelin or the adhesion molecules after 120 minutes of the infliximab infusion (data not shown).

Discussion

The present study shows that in patients with severe RA, refractory to conventional DMARD therapy, on periodical treatment with anti-TNF-α-blocker infliximab and ongoing disease activity, there is no correlation between serum leptin levels and most clinical and laboratory parameters of disease activity and inflammation. However, serum leptin levels positively correlated with BMI. Also, this study shows for first time that there is no change in serum leptin levels upon anti-TNF-α infliximab infusion.

Metabolic syndrome features are independently associated with atherosclerosis in RA (24, 25). However, in contrast to what was reported in non-RA subjects, only BMI but not insulin resistance, blood pressure or the lipid profile was related to leptin concentrations in this study.

A recent report on 23 patients with RA disclosed a correlation of IL-1 receptor antagonist with leptin independent of age and BMI (26). This study supported a potential relationship between inflammation and leptin. In keeping with these observations, in a series of 31 patients with RA starting either anti-TNF-α treatment or placebo, Popa *et al*. found that leptin concentrations at baseline correlated inversely with the degree of inflammation as assessed by CRP and IL-6 (27). However, in a series of 37 RA patients with disease duration greater than 10 years, Targońska-Stępniak *et al*. found that besides a positive correlation between leptin levels and BMI, leptin concentrations were significantly higher in patients with

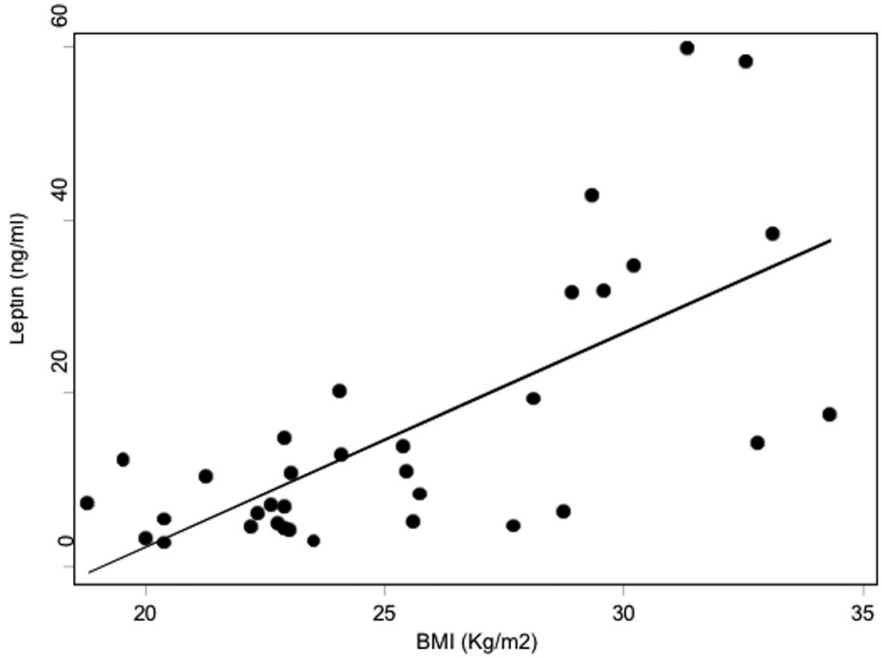

Fig. 1. Correlation between basal leptin concentrations and BMI in 33 patients with severe RA on periodical treatment with anti-TNF-α therapy.

higher disease activity (DAS28> 5.1) than in those with DAS28 ≤5.1 (28).

Taking into account the studies discussed above, a potential explanation for differences between our results and those by Popa *et al*. might be the degree of disease activity in RA patients. However, in our series of severe RA patients periodically treated with anti-TNF-α therapy, we did not observe significant differences in leptin concentrations when patients were stratified by disease severity at the time of the study as reflected by a DAS28 of >5.1 versus of ≤5.1 or the CRP concentrations prior to infliximab infusion.

However, in agreement with our results, in a series of 54 patients with RA, Gunaydin *et al*. did not observe correlations between serum leptin levels and disease duration, swollen and tender joint counts, DAS28, CRP, ESR, oral glucocorticoid and methotrexate usage (29). In addition, unlike Targońska-Stępniak *et al*. (28), these authors did not find statistically significant serum leptin level difference between patients with high disease activity and mild and low disease activity. Also, as reported in our study, serum leptin levels were higher in women than men and they positively correlated with BMI (29).

We previously reported a prompt change in resistin (17) but not in adiponectin concentrations (16) following infliximab infusion in RA patients with severe disease. The present study shows for first time that there is no significant change in leptin concentration following an anti-TNF-α blocker- infliximab infusion. These results are in keeping with those of Popa *et al*. who found an absence of change plasma leptin concentrations after 2 weeks' treatment with the use of the fully humanized anti-TNF-α blocker adalimumab (27). Similarly, Härle *et al*. reported no significant change of serum concentrations of leptin and adiponectin during 12 weeks treatment with adalimumab in patients with RA (30).

Finally, we have recently found that TNF-α blockade in patients with RA results in increased circulating ghrelin concentrations and that the latter is associated with decreased endothelial activation (22). Ghrelin has been associated with metabolic syndrome features (31) and ghrelin administration has beneficial effects not only on cachexia in patients with heart failure and chronic obstructive pulmonary disease (32) but also on insulin sensitivity in overweight patients and endothelial dysfunction in patients with the metabolic syndrome (33). However, in contrast to what was reported in non-RA subjects, metabolic syndrome features were not related to ghrelin concentrations in our series of patients with severe RA (22). Also, in the present investigation on the same cohort of RA patients, leptin concentrations did not correlate with ghrelin concentrations before and after TNF-α blockade.

In conclusion, in RA patients with severe disease on periodical treatment with anti-TNF-α blocker infliximab, there is no correlation between serum leptin levels and clinical and laboratory parameters of disease activity. However in these patients with severe and active disease, serum leptin levels positively correlate with BMI. In addition, serum leptin concentrations do not show significant changes upon infliximab administration.

Overall, in RA patients on TNF-α blocker treatment circulating leptin concentrations are unrelated to disease activity but constitute a manifestation of adiposity as in non-RA subjects. Moreover, the beneficial effect of anti-TNF-α therapy on the enhanced CV mortality observed in RA does not seem to be mediated by a reduction in serum levels of leptin.

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