Leptin Serum Levels Are Not Correlated With Disease Activity in Patients With Rheumatoid Arthritis

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Leptin, the *ob* gene product, has been proposed as a mediator of inflammatory cytokine–dependent decreased food intake and cachexia in rodents. In humans, leptin serum levels increase after administration of tumor necrosis factor-alpha (TNF-α) or interleukin-2 or during septicemia. However, the effect of human chronic inflammatory disease on serum leptin is unknown. We therefore determined the serum leptin level (radioimmunoassay), body mass index (BMI), percent body fat ([%BF] bioelectrical impedance analysis), and disease activity (Disease Activity Score [DAS]) in 58 patients with rheumatoid arthritis (RA) and 16 controls. The BMI, %BF, serum leptin, and ratio of leptin to %BF (leptin/%BF) did not differ significantly in 25 patients with moderate RA activity (DAS, 3.6 ± 0.5), 33 patients with low RA activity (DAS, 1.8 ± 0.5), and controls. A positive correlation for serum leptin and %BF was detected in all groups. Our data indicate that in RA, a human chronic cytokine-mediated inflammatory disease, the serum leptin level is directly related to %BF but not to disease activity.

THE PROTEIN LEPTIN is synthesized by adipose tissue as the product of the *ob* gene.[1,2] In mice, mutations in the *ob* gene cause obesity due to a lack of circulating leptin, and administration of recombinant leptin is followed by substantial weight loss.[3-5] In humans, the relevance of leptin in the regulation of appetite, food intake, and body weight is still subject to debate. Leptin serum levels have been found to be exclusively associated with gender and body fat content (percent body fat [%BF]), but appear not to depend on diet composition, fasting, feeding, estrogens, or physical activity.[6-14] However, in rodents and humans, serum leptin levels and food intake change following administration of inflammatory cytokines such as interleukin-1 and tumor necrosis factor-alpha ([TNF-α] cachectin), suggesting that leptin may be a mediator of anorexia in cytokine-mediated chronic diseases.[15-20] This hypothesis is further supported by data from recent studies that report increased serum leptin in patients with acute sepsis and after major surgery, although elevated serum leptin levels were not found in patients with acquired immune deficiency syndrome, chronic inflammatory bowel disease, or cancer.[21-26]

Rheumatoid arthritis (RA) is a chronic systemic inflammatory disease of the synovial tissue. Increased serum levels of inflammatory cytokines have been found to correlate with RA activity and progression.[27-29] Furthermore, cachexia and reduced lean body mass are commonly observed in RA, and have been related to high serum and synovial levels of inflammatory cytokines.[30]

To assess whether serum leptin levels are elevated in chronic rheumatic disease, we examined the relationship between serum leptin and disease activity in patients with RA.

SUBJECTS AND METHODS

Patients

Between June and September 1997, 58 patients (aged 25 to 79 years) with classic RA according to current American College of Rheumatology criteria were randomly recruited at our institution. Subjects who met one of the following criteria were excluded from the study: unstable weight (<1-kg change in 4 weeks), history of diabetes mellitus, or use of medications known to affect nutritional status or regulation of fat metabolism. Since the study was based on a one–time point evaluation of disease activity, any regular disease-modifying antirheumatic drug for RA therapy was allowed. The medications included low-dose steroids (57%), low-dose methotrexate (35%), sulfasalazine (35%), aureamine and azathioprine (one patient each), and chloroquine (two patients). Four patients (7%) were taking nonsteroidal antiinflammatory drugs only. Sixteen healthy subjects aged 33 to 81 years and not taking any medication served as a control population. All subjects provided written informed consent for the study. The number of patients enrolled was determined by assuming a 20% difference in the serum leptin concentration versus %BF. To detect a difference of this magnitude at an α level of .05 and β level of 0.2, respectively (80% power), 10 patients in each group were required.

RA activity was determined using the Disease Activity Score (DAS), an established measure for the evaluation of current RA activity.[31] Relevant parameters of the score include the following: total number of tender joints of 53 joints (Ritchie Articular Index [RAI]), swollen joint count of 44 joints (TSJI), erythrocyte sedimentation rate in millimeters per hour (ESR), and general health self-assessment by marking a 100-mm visual analog scale (GHA). The DAS was calculated using the following formula: $DAS = 0.53938 \times \sqrt{RAI + 0.06465 \times TSJI + 0.33 \times \ln ESR + 0.00722 \times GHA}$. Subjects were divided into the following three groups: controls, patients with low RA activity (DAS < 2.7), and patients with moderate RA activity (DAS > 2.7).

Laboratory Methods

%BF was determined using bioelectric impedance analysis (Akern-RJL BIA 101/S; Data Input, Frankfurt, Germany) in the fasting state as reported previously.[32,33] For measurement of serum leptin, venous blood samples were collected between 8 and 9 AM after an overnight fast, and sera were kept frozen at −80°C until analysis. Serum leptin concentrations were measured using a radioimmunoassay (Linco Research, St. Charles, MO) with a sensitivity of 0.5 ng/mL. The normal fasting range for serum leptin is 3.8 ± 1.8 ng/mL in lean men (n = 25) and 7.4 ± 3.7 ng/mL in lean women (n = 31). The within- and between-assay coefficient of variation for repeated measurements of five human sera with a varying leptin concentration was consistently less than 5.2% and 4.9%, respectively.

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Table 1. Characteristics, Serum Leptin Concentration, and %BF of 58 RA Patients and 16 Controls

Parameter	Intergroup Comparison			Intragroup (RA patients) Comparison		
	RA Patients	Controls	*P*	DAS <2.7 (n = 39)	DAS ≥2.7 (n = 25)	*P*
Age (yr)	59 ± 12	55 ± 15	.3	57 ± 14	63 ± 9	.1
Duration of RA (yr)	9.5 ± 9.5	—	—	5.3 ± 9.2	5.9 ± 9.8	.4
DAS	2.5 ± 0.5	—	—	1.8 ± 0.5	3.6 ± 0.5	<.00001
Body weight (kg)	66 ± 12	72 ± 16	.13	68 ± 12	65 ± 14	.2
BMI (kg/m²)	24 ± 4	25 ± 4	.29	24 ± 3	24 ± 4	.5
%BF	25 ± 7	25 ± 5	.85	24 ± 6	26 ± 7	.24
Leptin (ng/mL)	13.7 ± 9.4	11.3 ± 5.9	.54	11.3 ± 6.9	17.8 ± 12.7	.1
Leptin/%BF	0.51 ± 0.29	0.48 ± 0.31	.87	0.46 ± 0.25	0.61 ± 0.37	.22
ESR (per hour, mm)	37 ± 21	10 ± 7	.008	15 ± 10	55 ± 22	.00001

NOTE. Data are the mean ± SD.

Statistical Analysis

Data are presented as the mean ± SD. *P* values were determined by Student's *t* test. Correlations were determined by linear regression analysis (SAS software version 6.1. for Windows; SAS Institute, Cary, NC).

RESULTS

Characteristics of the patients are summarized in Table 1. Controls and patients with RA did not differ significantly with respect to weight, body mass index (BMI), %BF, serum leptin, and leptin/%BF. For RA activity assessed by the DAS, body mass parameters (weight, BMI, and %BF) and serum leptin levels were not significantly different in patients with low disease activity (DAS < 2.7) compared with patients with moderate disease activity (DAS > 2.7). Furthermore, although there was a significant correlation between serum leptin and %BF in all groups ($r = .882$, $r = .705$, and $r = .593$ for controls, patients with DAS < 2.7, and patients with DAS > 2.7, respectively), regression analysis of the relationship between serum leptin and %BF failed to show a significant difference between the groups (Fig 1).

DISCUSSION

The results of our study demonstrate that in RA, serum leptin concentrations are not significantly different from those in healthy subjects with a similar body fat content and BMI. Serum leptin levels were not altered by the degree of clinical activity of RA, but were directly related to the absolute amount of body fat in all groups. This confirms the observation that adipose tissue–derived leptin in serum is closely associated with the amount of body fat, and may act as an afferent signal to the brain reflecting the size of the adipose tissue depot.[8] Although serum cytokine levels were not measured in this study, our data are consistent with reports by others who measured leptin concentrations in chronic human cytokine–mediated disorders such as chronic viral infection, inflammatory bowel disease, and malignancy.[23-26] The finding that body fat–corrected serum leptin levels are only increased in mice with bacterial peritonitis and in humans with sepsis, following major surgery, and after administration of inflammatory cytokines suggests that serum leptin levels are elevated in acute, but not in chronic, inflammatory disease.[19-22,34] As a short-acting inflammatory cytokine,

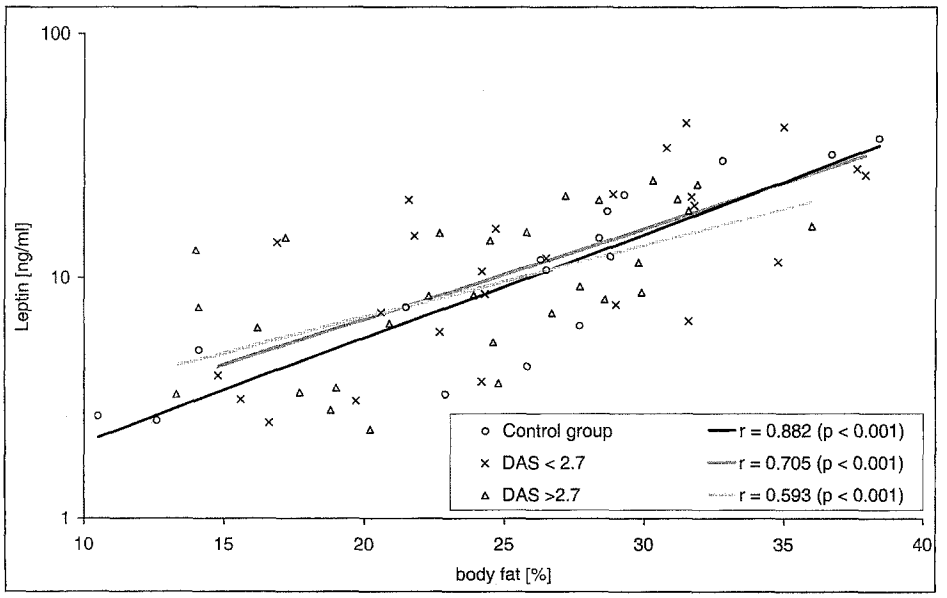

Fig 1. Correlation between %BF and serum leptin in controls ($r = .882$, $y = 0.043x + 0.19$), RA patients with DAS < 2.7 ($r = .705$, $y = 0.037x + 0.06$), and RA patients with DAS > 2.7 ($r = .593$, $y = 0.029x + 0.24$).

TNF-α has been shown to be a regulator of *ob* gene expression.[16] The relevance of TNF-α in the pathogenesis and systemic features of active RA has also been clearly demonstrated by recent advances in RA therapy with TNF-α antagonists.[35,36]

However, a persistent elevation of cytokine levels in active rheumatic disease seems not to have an effect on leptin secretion similar to the occasional TNF-α peak levels after experimental injection. The functional effects of secreted inflammatory cytokines depend strongly on interactions among different paracrine secreted cytokines and inflammatory mediators. Therefore, a sustained elevation of inflammatory cytokines in chronic arthritis may not necessarily induce sustained leptin secretion, because TNF-α may inhibit leptin secretion via TNF-α receptor type 1 in the absence of transforming growth factor-beta.[37,38] Furthermore, we cannot exclude that certain disease-modifying antirheumatic agents and corticosteroids used for the treatment of RA directly altered leptin secretion. However, a recent study indicates that leptin plasma levels do not change in patients with Cushing's disease shortly after surgical correction of hypercortisolism, suggesting that in humans corticosteroids do not alter leptin secretion independently of a steroid-related increase in %BF.[39]

It is possible that antirheumatic treatment decreased the assumed proinflammatory stimulus for aberrant leptin secretion in our patients by limiting the disease activity via a reduction of local cytokines.[40] However, patients with a DAS of 3.6 ± 0.5 still had substantial clinical and biochemical disease activity (elevated ESR) despite therapy.[31] Although we failed to detect a statistically significant difference in leptin levels between healthy subjects and patients with RA, we cannot exclude that leptin is involved in the metabolic regulation of chronic inflammatory disease. Cytokine-mediated effects on leptin secretion may be mitigated in patients with chronic rheumatic disease. Thus, a larger sample of untreated patients will be required to address this issue.

In conclusion, serum leptin levels correlate with %BF but are not altered in patients with mild to moderate RA disease activity compared with healthy controls.

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