

Leptin and adiponectin levels in patients with ankylosing spondylitis. The effect of infliximab treatment

C.S. Derdemezis¹, T.D. Filippatos¹, P.V. Voulgari², A.D. Tselepis³, A.A. Drosos², D.N. Kiortsis¹

¹Laboratory of Physiology, Medical School, University of Ioannina,

²Rheumatology Clinic, Department of Internal Medicine, Medical School, University of Ioannina, ³Laboratory of Biochemistry, Department of Chemistry, University of Ioannina, 45110 Ioannina, Greece.

Christos S. Derdemezis, BSc
Theodosios D. Filippatos, MD

Paraskevi V. Voulgari, MD
Alexandros D. Tselepis, MD, PhD
Alexandros A. Drosos, MD, FACR
Dimitrios N. Kiortsis, MD, PhD

Please address correspondence to:
Alexandros A. Drosos, MD, FACR,
Rheumatology Clinic,
Department of Internal Medicine,
Medical School, University of Ioannina,
45110 Ioannina, Greece.

E-mail:

adrosos@cc.uoi.gr; www.rheumatology.gr

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ABSTRACT

Background. Adipose tissue-derived leptin and adiponectin control hunger, energy expenditure, insulin sensitivity, endothelial function, reproduction and immunity and are thought to play a role in autoimmune diseases. However, their role in ankylosing spondylitis (AS) is not clearly defined. Tumour necrosis factor TNF- α is a potential modulator of adipocytokines. The effect of long-term anti-TNF- α treatment on plasma levels of leptin and adiponectin has not been assessed so far.

Objectives. To assess the effect of a 6-month anti-TNF- α treatment on serum leptin and adiponectin levels in AS patients.

Methods. Thirty men with AS were included in the study. Thirty age- and weight-matched men served as controls. Clinical and biochemical parameters were assessed and serum levels of leptin and adiponectin were measured with enzyme immunoassay methods prior to and after the 6-month treatment with infliximab.

Results. Mean age and disease duration of AS patients were 40.6 ± 13.7 and 13.4 ± 8.4 years, respectively. At baseline, AS patients exhibited significantly higher adiponectin (15.4 ± 8.3 vs. 8.6 ± 4.2 $\mu\text{g/ml}$, $p < 0.05$), but no difference in leptin levels (7.2 ± 2.9 vs. 8.9 ± 6.4 ng/ml , $p = \text{NS}$). Adipocytokines did not correlate with any disease parameter. Body weight of the patients did not change significantly over the 6-month period. Serum levels of leptin and adiponectin did not change significantly after the 6-month treatment.

Conclusion. Adiponectin levels were significantly higher in AS patients compared with controls. Infliximab treatment did not change serum levels of leptin and adiponectin suggesting that the anti-TNF- α treatment may not modulate significantly their levels.

Introduction

Our understanding of the physiology of adipose tissue has grown in recent years and is now considered to be an active endocrine organ, as opposed to solely being fat storage tissue. Its products, collectively referred to as adipocytokines, are implicated in appetite

and hunger control, energy expenditure, insulin sensitivity, endothelial function, reproduction and immunity. Of these, leptin and adiponectin are the most abundant and have attracted the most interest (1, 2).

Leptin is predominantly produced from adipocytes and its primary role is appetite control. Mice lacking leptin or its receptor are massively obese, yet in humans obesity correlates with circulating leptin. It has also been found to possess a role in lipoprotein metabolism, acute phase reactants, sex hormones and glucocorticoid metabolism, and immune function. With regard to immunity and inflammation, it protects T lymphocytes from apoptosis, regulates their proliferation and activation, and influences monocyte activation, phagocytosis and cytokine production (1, 2).

Adiponectin is a 30-kD protein that shares structural homology to tumour necrosis factor TNF- α and is also mainly synthesised by adipocytes (3). It acts as an anti-diabetic and anti-atherogenic adipocytokine. Levels of adiponectin in the blood are decreased under conditions of obesity, particularly visceral obesity, insulin resistance and type-2 diabetes. It inhibits NF- κB activation in endothelial cells, interferes with the macrophage function and induces production of anti-inflammatory cytokines such as IL-10 (1, 2).

Interestingly, leptin and adiponectin levels have been proposed to possess a role in autoimmune diseases, such as rheumatoid arthritis (RA), Crohn's disease, inflammatory bowel disease and lupus (1, 2, 4). Ankylosing spondylitis (AS) is a chronic, progressive, inflammatory spondyloarthropathy affecting primarily the sacroiliac joints and axial skeleton. It is a dissembling disease causing spinal ankylosis and kyphosis (5, 6). Anti-TNF- α treatment has been proven particularly beneficial in AS and is established as the treatment choice (7). Furthermore, it confers additional benefits such as endothelial function (8) and insulin resistance (9).

Evidence exists for a cross talk between adipocytokines and TNF- α (1, 2). Its production is influenced by leptin and adiponectin: leptin increases the

Competing interests: none declared.

Table I. Clinical and biochemical characteristics of study population before and after 6 months of infliximab treatment.

Parameter*	Controls (n=30)	AS (n=30)		p (vs. baseline)
		Baseline	6 months	
Age (years)	41.7 ± 12.4	40.6 ± 13.7	–	–
Weight (kg)	76.2 ± 12.1	77.3 ± 9.2	78.9 ± 8.5	NS
BMI (kg/m ²)	25.1 ± 3.6	24.7 ± 4.3	25.1 ± 3.8	NS
Waist circumference(cm)	93.0 ± 3	92.5 ± 3	93.0 ± 2	NS
Disease duration (years)	–	13.4 ± 8.4	–	–
Leptin (ng/ml)	8.9 ± 6.4	7.2 ± 2.9	8.5 ± 4.1	NS
Adiponectin (µg/ml)	8.6 ± 4.2**	15.4 ± 8.3	15.4 ± 7.8	NS
TC (mg/dl)	222 ± 49**	201 ± 39	207 ± 42	NS
HDL-C (mg/dl)	40 ± 10**	46 ± 8	48 ± 12	NS
Triglycerides (mg/dl)	124 ± 47	101 ± 48	117 ± 52	NS
LDL-C (mg/dl)	157 ± 46**	132 ± 33	141 ± 34	NS
Glucose (mg/dl)	95 ± 12	90 ± 9	91 ± 10	NS
BASDAI	–	5.5 ± 1.4	1.8 ± 0.9	<0.01
BASFI	–	65.5 ± 19.6	32.0 ± 16.8	<0.01
BASMI	–	4.5 ± 0.9	4.3 ± 0.8	NS
CRP (mg/l)	4 ± 2**	24 ± 14	12 ± 11	<0.01
ESR (mm/1st h)	8.3 ± 3**	32 ± 22	21 ± 17	<0.01

*mean ± SD; **p<0.05 compared to AS baseline

production of TNF- α , the former suppresses the transcription of adiponectin in adipocytes and treatment of cultured macrophages with adiponectin markedly inhibited their phagocytic activity and production of TNF- α in response to stimulation with lipopolysaccharide. So far few studies have evaluated serum levels of leptin and adiponectin in AS, and no data exist with respect to the effect of anti-TNF treatment on serum levels of leptin and adiponectin. The present study has sought to determine serum levels of leptin and adiponectin in AS patients and, for the first time, the effect of 6-month infliximab treatment on their levels in these patients.

Patients and methods

Participants

Thirty consecutive patients with AS, followed in the outpatient Rheumatology Clinic of the University Hospital of Ioannina, were included in the study. All were receiving non-steroidal anti-inflammatory drugs, while two were receiving sulfasalazine. The dose of the drugs remained stable during the study. In all patients infliximab was administered (5mg/kg of body weight) at 0, 2, 6 weeks and every 8 weeks thereafter for a period of 6 months. At each visit complete physical evaluation was assessed and laboratory tests were performed including complete blood

count and differential, serum glucose, total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), triglycerides, creatinine, as well as liver function test and urine analysis. Finally, C-reactive protein (CRP) and erythrocyte sedimentation rate (ESR) were also performed. AS disease activity was evaluated using the Bath Ankylosing Spondylitis Disease Activity Index (BASDAI) (10), the Bath Ankylosing Spondylitis Functional Index (BASFI) (11) and the Bath Ankylosing Spondylitis Metrology Index (BASMI) (12). Exclusion criteria were: (a) a history or presence of malignant disease; (b) known liver or kidney abnormalities or history of viral hepatitis B and C; (c) major complicating diseases such as amyloidosis, heart or lung disease; (d) diabetes mellitus; (e) endocrine or metabolic disorders; (g) a positive tuberculin skin test or abnormal chest x-ray findings; and (h) previous anti-TNF- α treatment or administration of drugs which might influence glucose and lipid metabolism.

Thirty healthy age- and weight – matched male volunteers, fulfilling the same exclusion criteria, from hospital staff constituted our control group. All participants reported no significant change in their body weight for at least 3 months before entry into the study and modification of dietary habits dur-

ing the study, as evaluated by a registered dietician using personal interview and food frequency questionnaires. Written informed consent was obtained from all subjects and the study protocol was approved by the Institutional Ethics Committee.

Biochemical parameters

All laboratory determinations were carried out after overnight fast with methods previously described (13). Serum concentrations of fasting glucose, TC, triglycerides, liver enzymes and creatinine were determined enzymatically on an Olympus AU600 clinical chemistry analyser (Olympus Diagnostica). HDL-C was determined in the supernatant, after precipitation of the apolipoprotein B-containing lipoproteins with dextran sulphate-Mg⁺⁺ (Sigma Diagnostics). Low-density lipoprotein cholesterol (LDL-C) was calculated using the Friedewald formula (provided that triglycerides were <3.9mmol/L, 350mg/dL). Serum CRP concentrations were measured by a high sensitive immunonephelometric assay (Beckman Instruments).

Measurement of leptin and adiponectin

Serum leptin and adiponectin levels were determined by specific ELISA assays using material and protocols supplied by the provider (BioVendor, Heidelberg, Germany). Intra-assay and inter-assay coefficient of variation were 6.7% and 8.1% for leptin and 6.6% and 7.5% for adiponectin, respectively. The limits of detection for leptin and adiponectin immunoassays were 0.17–50ng/ml and 0.7–100µg/ml, respectively, according to manufacturer. Samples exceeding these values were repeated after appropriate dilution.

Statistical analysis

Values are given as mean ± standard deviation (SD). The paired-samples t-test was used for assessing the effect of treatment. Correlations were assessed with the Pearson correlation coefficients accordingly. Significance was defined as p<0.05. Analyses were performed using the SPSS 12.0 statistical package for Windows (SPSS Inc., 1989–2004).

Results

Patients' age was 40.6 ± 13.7 years with disease duration of 13.4 ± 8.4 years. All AS participants had active disease as evaluated by high BASDAI, CRP and ESR (Table I). Leptin levels did not differ between controls and AS (8.9 ± 6.4 vs. 7.2 ± 2.9 ng/ml, $p > 0.05$). Adiponectin was higher in AS patients compared to controls (8.6 ± 4.2 vs. 15.4 ± 8.3 , $p < 0.05$). TC and LDL-C were significantly lower in AS patients and HDL-C, CRP and ESR higher (Table I). Neither leptin nor adiponectin correlated with any disease parameter.

The patients exhibited a significant favourable clinical and metabolic response during anti-TNF- α treatment: BASDAI, BASFI and CRP levels were significantly reduced (14). No changes on BASMI were noted. No significant change in body weight or lipid parameters was observed after treatment (Table I). Serum leptin and adiponectin levels did not change significantly in the whole study population during treatment.

Discussion

In the present study serum levels of leptin and adiponectin were measured in AS patients before and after six-month treatment with infliximab. Adipocytokines, apart from energy homeostasis regulators, are now widely regarded as modulators of immune response and possess a potentially major role in various pro-inflammatory and inflammatory conditions, such as obesity, diabetes, inflammatory bowel disease, asthma and RA (1, 2). We observed no difference in leptin levels between controls and AS. In contrast, adiponectin levels were found higher in AS patients.

Sari *et al.* (15) reported lower leptin levels in patients with AS, both males and females. The study group, though, was younger than ours (median age 30 years), and AS male participants had very low body fat percentages that could significantly downregulate leptin levels, since adipose tissue is the major source of circulating leptin. Another group found higher leptin levels in subjects with AS and a correlation with disease parameters (16). Further analysis from that group suggested that

peripheral blood mononuclear cells from AS patients could explain higher leptin levels (17). Finally, Toussirot *et al.* (18) have also examined leptin levels in AS and reported increased leptin levels, yet with values in patients similar to ours. These discrepancies could be attributed to differences in race, sex, age, age at disease onset, disease duration, body mass index, cardiovascular disease risk factors and medication and thus account for the fact that our results are not in agreement with previous studies.

We obtained significantly higher adiponectin levels in AS patients. The results of the present study, showing higher adiponectin concentrations, favour adiponectin as an inflammatory index in AS. However, we did not find any correlation between adiponectin and disease parameter or inflammation. Another group (18) reported no significant difference in adiponectin levels between AS patients and controls. The role of adiponectin in auto-inflammatory conditions is still under investigation. However, the fact that we, as well as others (4, 19, 20), showed circulating adiponectin to be significantly elevated in RA, another autoimmune disease affecting primarily the skeleton, suggests that this might also be the case in AS. Recent research has identified adiponectin as a possible link between low adiposity and increased radiographic damage in RA (21). Adiponectin may drive arthritis via stimulation of vascular endothelial growth factor and metalloproteinase-1 and -13 in fibroblast-like synoviocytes (22). Hence, some support the notion that adiponectin may represent a novel strategy for attenuating articular damage (21). The cross talk between TNF- α and adipocytokines provides a thorough theoretical basis for the investigation of anti-TNF therapy effect on adipocytokine levels. In the present study, anti-TNF- α treatment led to significant clinical improvement of our patients, but no change in plasma levels of any of the two adipocytokines was observed after anti-TNF- α treatment. We and others (4, 23, 24) have addressed the same question in RA. Similarly, neither leptin, nor adiponectin were changed after anti-TNF

treatment, which suggests that anti-TNF therapy may not be an important modulator of adipocytokine levels. Regrettably, and because the purpose of the study was the long-term effect of anti-TNF treatment, we did not obtain samples and measurements during the study. However, a study by Harle *et al.* (25), showed no variation in adipokine levels during the whole study period of 3 months in RA patients treated with another anti-TNF agent. We cannot exclude, though, a possible variation in AS patients, and this requires further investigation.

In conclusion, adiponectin, but not leptin, is increased in AS patients. Recent evidence suggests involvement of adipokines in joint diseases and indicates that they may mediate inflammation (26). However, we found no correlation between adiponectin levels and inflammatory markers (ESR, CRP, BASFI). This contradiction requires further investigation. A 6-month infliximab treatment improved clinical conditions and biochemical parameters, whereas no changes in leptin and adiponectin levels were observed. Studies with larger number of patients may be necessary to further explore the possible role of adipocytokines in AS, as well as the role of anti-TNF- α therapy in adipocytokine physiology in AS.

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