Clinical associations of serum leptin and leptin/adiponectin ratio in systemic sclerosis

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Abstract
Introduction: Leptin and adiponectin have recently received the attention of researchers as attractive biomarkers in systemic sclerosis (SSc) because of their role in the inflammatory process, vascular function and fibrosis. We hypothesized that leptin and adiponectin may be associated with disease activity and severity in patients with SSc.

Aim: To compare serum leptin, adiponectin and leptin/adiponectin levels in patients with SSc and healthy controls and to evaluate their possible relationship with frequently used laboratory markers and clinical findings.

Material and methods: The study included 48 Caucasian female patients with SSc and 38 healthy controls. Serum concentrations of leptin and adiponectin were measured in patients and controls using commercially available ELISA Kits (Quantikine ELISA Kit R&D Systems, Minneapolis, MN, USA). The results were assessed by the Mann-Whitney U-test and Spearman’s correlation test.

Results: Leptin and adiponectin levels correlated with body mass index in SSc patients (r = 0.495, p = 0.000398 and r = −0.306; p = 0.0342) in contrast to healthy controls (p = 0.070 and p = 0.256, respectively), and, in SSc patients only, a strong negative correlation was observed between leptin and adiponectin serum levels (r = −0.314; p = 0.0312). Diffuse form of the disease (dcSSc) was associated with significantly lower serum adiponectin levels (8638.62 ±10382.62). Active disease was associated with significantly lower leptin concentration (13700.49 ±18293.32) and there was a significant negative correlation between leptin serum level and activity index score (r = −0.342; p = 0.0185).

Conclusions: The results of our study indicate that leptin levels might correlate with disease activity and subtype in SSc patients.

Key words: leptin, adiponectin, systemic sclerosis, disease activity.

Introduction
Systemic sclerosis (SSc) is a multiorgan connective tissue disease characterized by vascular injury and chronic autoimmune inflammation followed by excessive extracellular matrix deposition and ultimately progressive fibrosis of skin and internal organs. The etiology of SSc is still obscure, although genetic and environmental factors seem to underlay its pathogenesis [1]. In recent years, mediators synthesized in the adipose tissue, called adipocytokines, have arisen as new players in modulating immune responses and have been reported to play important roles in the pathogenesis of autoimmune rheumatic diseases [2–7].

Adipocytokines (or adipokines) are biologically active hormones secreted by adipocytes. Notably, two members of this family, namely leptin and adiponectin, seem to be attractive, since they are found to have opposing action. It is generally accepted that in most inflammatory diseases leptin displays an enhancing effect in inflammatory and immune responses, while adiponectin is considered to act primarily as an anti-inflammatory molecule [2, 3, 6, 8, 9].

Leptin, a 16-kD non-glycosylated polypeptide, is a cytokine-like hormone, synthesized mainly by the adipose tissue cells and primarily involved in regulating food intake, basal metabolism and body weight [3, 5, 10, 11]. Thus, the serum concentration of this hormone depends on the percentage of adipose tissue (it is positively cor-
related with body mass index (BMI)), but also on the patient's age and sex [6, 12]. It structurally and functionally resembles the interleukin 6 (IL-6) cytokine family and binds to the receptor that is a member of the class I cytokine receptor family [3, 10–12]. In the recent years, it has become apparent that the leptin receptor is widely expressed in all cell types of innate and adaptive immunity [13–16]. The interactions between leptin and inflammation are bidirectional. Leptin synthesis in humans shows a detectable increase as a response to acute inflammation, and secretion of inflammatory mediators such as IL-1, IL-6 and tumor necrosis factor-α (TNF-α). Leptin, in turn, up-regulates the production of pro-inflammatory cytokines such as IL-6, IL-12, and TNF-α, thus perpetuating the loop of inflammation. In innate immunity, leptin also activates proliferation of monocytes, macrophages, chemotaxis of neutrophils, maturation of dendritic cells and cytotoxicity of natural killer cells (NK) [3, 5, 6, 10, 11, 13, 14, 17, 18]. In adaptive immune responses, leptin may exert direct effects on lymphocytes. It stimulates secretion and maturation of thymocytes and T cell proliferation and polarizes TH0 towards a TH1 response with production of pro-inflammatory cytokines such as interferon γ (INF-γ) and IL-2 [13, 14, 19, 20]. Regarding B cells and immunoregulation, leptin activates them to produce cytokines such as IL-6, IL-10 and TNF-α [21]. Furthermore, leptin increases expression of adhesion molecules such as intercellular adhesion molecule-1 (ICAM-1) and very late antigen-2 (VLA2), which contributes to activation and migration of the cells to the inflammation site [13, 22]. As a result, TH1 and B cells' immune response stimulation may lead to the development and progression of autoimmune processes. Moreover, leptin is able to reduce the human CD4+CD25+ Treg cells, which are a small subset of CD4+ T cells that control the peripheral immune tolerance, and prevent inappropriate immune responses, such as allergy and autoimmunity [13, 14]. In fact, experimental investigations on animal models have clearly indicated that leptin can promote autoreactivity [13]. In animals with adaptive immunity-mediated inflammation antigen-induced arthritis, or other autoimmune diseases, leptin deficiency has a protective effect by resulting in reduced production of proinflammatory Th1 cytokines and a shift towards a Th2 response [23]. Additionally, leptin-deficient ob/ob mice and leptin receptor-deficient db/db mice are resistant to the development of several experimentally induced autoimmune diseases [13].

Adiponectin is a biologically active protein built of 244 amino acids that form a trimer of 30 kDa weight [6, 7, 9]. Its structure is similar to collagen VIII and X as well as the C1q complement component. It is widely produced by adipocytes, but also by muscles, cardiac myocytes and endothelial cells [4]. Adiponectin acts via three receptors: AdipoR1 (expressed most abundantly in skeletal muscles), AdipoR2 (expressed in the liver) and T-cadherin (mainly found in the heart and arteries) [24, 25]. Opposite to leptin, adiponectin acts as a protective metabolic syndrome biomarker. Its levels are inversely proportional to obesity, BMI, N-terminal prohormone of brain natriuretic peptide (NT-proBNP), triglyceridemia and insulin resistance. It increases with weight loss and with use of insulin-sensitizing drugs [9]. At the site of inflammation, adiponectin seems to have anti-inflammatory action. It inhibits production of pro-inflammatory cytokines such as TNF-α, IL-6, IL-10, and IFN-γ; however, its secretion is down-regulated in response to pro-inflammatory cytokines and oxidative stress [8, 9, 25–30].

Taken together, we hypothesized that leptin and adiponectin through their wide range of molecular and immune properties may play a significant role in pathogenesis of systemic sclerosis. Although numerous studies have focused on circulating adiponectin and leptin levels in SSC patients, they brought inconclusive or contradictory results [2, 26, 31–42].

Aim

The aim of this study was to determine serum leptin and adiponectin levels in SSC patients according to healthy controls and to define possible correlations with clinical and laboratory profiles of SSC patients.

Material and methods

Patients

We recruited to the study 48 Caucasian female patients with SSC (aged 34–84 years, mean ± SD: 62 ±10.6 years), fulfilling the American College of Rheumatology (ACR) and/or EULAR classification criteria [18, 19]. The control group consisted of 38 healthy subjects, matched with patients for sex, age, BMI and race (aged 36–88, 56.3 ±9.7 years).

Patients and healthy controls were voluntarily recruited and informed consent was obtained from all participants. The study was approved by the Bioethics Committee. Patients with overlap syndromes, kidney disease, diabetes mellitus, thrombosis, metabolic syndrome/hyperlipidemia, pregnancy, neoplastic diseases and those with habitual cigarette smoking and alcohol drinking were excluded from the study.

Laboratory assessment

The material for the study was fasting peripheral blood drawn in the morning. The samples were allowed to clot for 30 min and centrifuged for 15 min at 1000 × g. Obtained sera were stored at −70°C immediately after collection till further analysis. Serum concentrations of leptin were measured using commercially available ELISA Kits (Quantikine ELISA Kit R&D Systems, Minneapolis, MN, USA) according to the manufacturer’s instructions. The concentration level of cytokine was calculated using...
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Clinical evaluations
Skin thickness was evaluated using a modified Rodnan skin score (mRSS) [43]. According to the criteria proposed by LeRoy et al., patients were classified as having either limited cutaneous SSC (lcSSc) or diffuse cutaneous SSC (dcSSc) [44]. The disease duration was measured from the onset of the first symptom, other than Raynaud phenomenon (RP), consistent with SSC. The disease activity was assessed according to the European Scleroderma Study Group (EScrSG) disease activity score for SSC (Valentini disease activity index) as active or inactive [45]. Microvascular abnormalities such as digital ulcers and osteolysis of the distal phalanges of the fingers (acroosteolysis) were also evaluated. Nailfold capillaroscopy was performed to estimate SSC microangiopathy and patients were classified into three groups presenting as an early, active or late pattern, according to the criteria proposed by Cutolo et al. [46]. Patients were evaluated with respiratory function tests (forced vital capacity – FVC, total lung capacity – TLC and diffusing capacity of the lungs for carbon monoxide – DLCO) and high-resolution computed tomography (HRCT). Patients with ground glass opacification, centrilobular nodules or a honeycomb picture were considered to have lung involvement. Pulmonary artery pressure and valvular insufficiency were assessed by color Doppler echocardiography (ECO). Pulmonary arterial hypertension (PAH) was defined as systolic pulmonary arterial pressure (sPAP) ≥ 35 mm Hg in Doppler echocardiography and was determined only at rest [47].

The presence of ANAs and their characteristics including anticientromere antibodies (ACAs), anti-topoisomerase 1 (anti-topo 1, Scl-70) antibodies, anti-RNA polymerase I or III antibodies, anti-U3- and U1-RNP, PM-Scl and anti-Ku antibodies was determined by means of indirect immunofluorescence on HEp-2 cells and/or immunoblot analysis.

Statistical analysis
The results were analyzed statistically using Statistica 10.0 PL software. Because the measurements were characterized by high skewness, the results were presented as the median, a measure of central tendency. Equality of distribution for each variable within normal distribution groups was tested using the Lilliefors version of the Kolmogorov-Smirnov test as well as the Shapiro-Wilk test. Because the test variables did not have a normal distribution, non-parametric tests were used for further analysis. These tests are resistant to deviations from the assumptions of normality of distribution and heterogeneity of variance in the groups compared. Pairs of independent groups were compared using the Mann-Whitney U test.

Results
Out of 48 patients, 42 had lcSSc and 6 had dcSSc. Mean disease duration was 12.85 ± 7.63 years. Limited

Table 1. Demographic and laboratory characteristics of patients with systemic sclerosis (SSc) and healthy control group

| Parameter                      | Patient group (n = 48) | Control group (n = 38) | Z   | p     |
|--------------------------------|------------------------|------------------------|-----|-------|
| Age, mean ± SD; range [years]   | 62.68 ±10.59; 34–84    | 56.35 ±9.87; 36–88     | 1.535 | 0.11  |
| Sex – female : male             | 48 : 0                 | 38 : 0                 |      |       |
| Disease duration, mean ± SD [years] | 12.85 ±7.6            |                        |      |       |
| Duration of RP, mean ± SD [years] | 16.91 ±9.3            |                        |      |       |
| BMI, mean ± SD [kg/m²]          | 26.57 ±4.42           | 25.51 ±3.38           | 1.347 | 0.177 |
| Total cholesterol, mean ± SD [mg/dl] | 197.62 ±34.32         | 202.06 ±30.27         | -0.634 | 0.52  |
| LDL, mean ± SD [mg/dl]          | 115.12 ±28.07         | 116.34 ±27.14         | -0.09 | 0.927 |
| HDL, mean ± SD [mg/dl]          | 57.39 ±19.69          | 62.79 ±12.80          | -1.895 | 0.058 |
| TG, mean ± SD [mg/dl]           | 127 ±44.23            | 104.36 ±35.40         | 2.630 | 0.008 |

RP – Raynaud phenomenon, BMI – body mass index, LDL – low-density lipoprotein, HDL – high-density lipoprotein, TG – triglycerides, SD – standard deviation.
cutaneous SSc was diagnosed in 42 patients, whereas the diffuse disease subtype was diagnosed among 6 SSc patients. All of the patients manifested RP, whereas 13 (27%) of them had active digital ulcers. When evaluated relative to the Valentini activity index, 9 (18.7%) patients had active disease and 39 (81.25%) had inactive disease. Furthermore, scleroderma-related interstitial lung disease (ILD), defined based on HRCT findings, was present in 43 (89.5%) patients and 11 (22.9%) SSc patients had elevated pulmonary artery pressure (sPAP ≥ 35 mm Hg) on ECO. Concerning cardiac involvement, 6 (12.5%) SSc patients had ECG changes and 31 (64.5%) had heart valves abnormalities. Considering the laboratory characteristics of SSc patients, the mean erythrocyte sedimentation rate (ESR) value in SSc patients was 26.065 ± 15.6 mm/h (range: 7–80/h), and the mean C-reactive protein (CRP) concentration was 7.94 ± 17.2 mg/l (range: 0.5–88.6). There was no significant difference in age, lipid profile values or BMI of SSc patients and those of the control group, except for higher TG values among SSc patients (range: 0.5–88.6). There was no significant difference in age, lipid profile values or BMI of SSc patients and those of the control group, except for higher TG values among SSc patients.

| Table 2. Correlation of leptin and adiponectin levels as well as their ratio with age and BMI among patients and healthy controls (Spearman test) |
|---------------------------------|----------------|----------------|----------------|----------------|
| **Correlation** | **Patient group** | **Control group** | **Control group** |
| | **R Spearman** | **P-value** | **R Spearman** | **P-value** |
| Age: | | | | |
| Leptin | −0.507 | 0.000276 | −0.190 | 0.258 |
| Adiponectin | 0.478 | 0.000586 | 0.116 | 0.491 |
| Leptin/adiponectin ratio | −0.583 | 0.000017 | −0.274 | 0.100 |
| BMI: | | | | |
| Leptin | 0.495 | 0.000398 | 0.296 | 0.070 |
| Adiponectin | −0.306 | 0.0342 | −0.188 | 0.256 |
| Leptin/adiponectin ratio | 0.488 | 0.000496 | 0.332 | 0.041 |
| Leptin: | | | | |
| Adiponectin | −0.314 | 0.0312 | −0.084 | 0.612 |

BMI – body mass index
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Table 3. Leptin and adiponectin serum levels as well as leptin/adiponectin, leptin/BMI and adiponectin/BMI ratios in patients with SSC and control group (Mann-Whitney U-test)

| Parameter                  | Patient group (n = 48) | Control group (n = 38) | Z     | p     |
|----------------------------|------------------------|------------------------|-------|-------|
| Leptin [pg/ml]             | 35025.02 ±37395.9      | 36939.5 ±30594.97      | –0.852, p = 0.393 |
| Adiponectin [ng/ml]        | 11873.61 ±8874.70      | 10803.4 ±5633.36       | –0.269, p = 0.787 |
| Leptin/adiponectin ratio   | 5.56 ±7.85             | 4.63 ±5.53             | –0.163, p = 0.870 |
| Leptin/BMI                 | 1242.22 ±1212.22       | 1444.23 ±1222.64       | –0.989, p = 0.322 |
| Adiponectin/BMI            | 475.64 ±408.93         | 431.27 ±241.58         | –0.547, p = 0.583 |

BMI – body mass index. Results are given as mean ± SD.

Table 4. Comparison of serum adipocytokine levels and ratios in SSC patients according to disease subtype and profile of antinuclear antibodies (Mann-Whitney U-test)

| Parameter                  | SSC patient group           |
|----------------------------|-----------------------------|
|                            | lcSSc (n = 42)              | dcSSc (n = 6)             |
| Leptin, mean ± SD [pg/ml]  | 36744.45 ±39159.91          | 23275.56 ±20196.15        |
|                            | Z = 0.733, p = 0.463        |                             |
| Adiponectin, mean ± SD [ng/ml] | 12425.92 ±8615.65 | 8638.62 ±10382.62        |
|                            | Z = 2.030, p = 0.042        |                             |
| Leptin/adiponectin ratio   | 5.14 ±7.35                 | 8.45 ±11.08               |
|                            | Z = –0.223, p = 0.823       |                             |
| Leptin/BMI                 | 1292.61 ±1261.00            | 897.87 ±796.88            |
|                            | Z = 0.765, p = 0.444        |                             |
| Adiponectin/BMI            | 496.29 ±401.86              | 354.71 ±461.88            |
|                            | Z = 1.884, p = 0.059        |                             |
| ACA positive (n = 26)      |                             |                             |
| Leptin, mean ± SD [pg/ml]  | 42417.62 ±44716.42          | 20497.58 ±17745.13        |
|                            | Z = 1.838, p = 0.066        |                             |
| Adiponectin, mean ± SD [ng/ml] | 12984.13 ±8763.01 | 10190.23 ±8241.17        |
|                            | Z = 1.408, p = 0.159        |                             |
| Leptin/adiponectin         | 5.651 ±8.378               | 4.567 ±7.158              |
|                            | Z = 0.894, p = 0.371        |                             |
| Leptin/BMI                 | 1473.83 ±1418.99            | 786.42 ±664.61            |
|                            | Z = 1.863, p = 0.062        |                             |
| Adiponectin/BMI            | 521.26 ±427.88              | 418.74 ±375.03            |
|                            | Z = 1.002, p = 0.316        |                             |

SD – standard deviation, lcSSc – limited cutaneous systemic sclerosis, dcSSc – diffuse cutaneous systemic sclerosis, BMI – body mass index, ACA – anticentromere antibodies, TopoI – anti-topo I (Scl-70) antibodies. Results are given as mean ± SD.

The levels of serum leptin, adiponectin, leptin/adiponectin, leptin/BMI and adiponectin/BMI ratios were not as...
Table 5. Correlation between leptin, adiponectin and leptin/adiponectin, leptin/BMI and adiponectin/BMI ratios with selected clinical and laboratory findings among SSc patients (Spearman’s rank correlation test)

| Parameter                  | Leptin   | Adiponectin | Leptin/Adiponectin | Leptin/BMI | Adiponectin/BMI |
|----------------------------|----------|-------------|--------------------|------------|----------------|
| ESR                        | $r = 0.106$ | $r = 0.156$ | $r = -0.139$      | $r = -0.119$ | $r = 0.150$    |
|                            | $p = 0.478$ | $p = 0.286$ | $p = 0.350$       | $p = 0.424$ | $p = 0.307$    |
| CRP                        | $r = 0.069$ | $r = -0.139$ | $r = 0.129$       | $r = 0.028$ | $r = -0.183$   |
|                            | $p = 0.640$ | $p = 0.345$ | $p = 0.384$       | $p = 0.851$ | $p = 0.212$    |
| mRSS                       | $r = -0.141$ | $r = -0.093$ | $r = -0.106$      | $r = -0.168$ | $r = -0.075$   |
|                            | $p = 0.344$ | $p = 0.525$ | $p = 0.478$       | $p = 0.258$ | $p = 0.608$    |
| Activity index             | $r = -0.342$ | $r = -0.047$ | $r = -0.303$      | $r = -0.370$ | $r = -0.024$   |
|                            | $p = 0.0185$ | $p = 0.748$ | $p = 0.037$       | $p = 0.010$ | $p = 0.868$    |
| C3                         | $r = 0.537$ | $r = -0.049$ | $r = 0.429$       | $r = 0.512$ | $r = -0.108$   |
|                            | $p = 0.00014$ | $p = 0.745$ | $p = 0.0032$      | $p = 0.000318$ | $p = 0.472$ |
| C4                         | $r = 0.340$ | $r = 0.046$ | $r = 0.201$       | $r = 0.302$ | $r = -0.035$   |
|                            | $p = 0.022$ | $p = 0.757$ | $p = 0.183$       | $p = 0.043$ | $p = 0.817$    |
| Disease duration [years]   | $r = -0.225$ | $r = 0.280$ | $r = -0.253$      | $r = -0.216$ | $r = 0.295$    |
|                            | $p = 0.128$ | $p = 0.053$ | $p = 0.065$       | $p = 0.143$ | $p = 0.041$    |
| DLCO                       | $r = 0.278$ | $r = 0.320$ | $r = -0.324$      | $r = -0.272$ | $r = 0.345$    |
|                            | $p = 0.177$ | $p = 0.109$ | $p = 0.113$       | $p = 0.186$ | $p = 0.083$    |
| TLC                        | $r = 0.163$ | $r = 0.155$ | $r = 0.076$       | $r = 0.164$ | $r = 0.137$    |
|                            | $p = 0.334$ | $p = 0.352$ | $p = 0.652$       | $p = 0.329$ | $p = 0.408$    |

ESR – erythrocyte sedimentation rate, CRP – C-reactive protein, mRSS – modified Rodnan skin score, BMI – body mass index, C3 – C3 complement concentration, C4 – C4 complement concentration, DLCO – diffusing capacity of the lungs for carbon monoxide, TLC – total lung capacity.

associated with disease duration, inflammatory markers, ESR and CRP as well as total skin thickness score (mRSS); however, we found a close to significant positive correlation of disease duration with serum adiponectin levels as well as a weak significant correlation with adiponectin/BMI ratio among studied patients with SSc ($r = 0.280; p = 0.053$) (Table 5).

When considering organ involvement, adiponectin serum levels as well as values of adiponectin/BMI ratio were significantly lower in patients with decreased FVC values (8598.92 ±1931.20 and 318.33 ±443.35, respectively) than in patients with normal FVC (12952.19 ±7981.97; $p = 0.046$ and 493.06 ±349.35; $p = 0.038$, respectively). Similarly, SSc patients with decreased TLC values had significantly lower adiponectin levels (6815.72 ±990.39) compared to SSc individuals with normal TLC (14102.66 ±9494.23; $p = 0.018$); although there was no significant correlation between serum adiponectin and TLC values ($r = 0.155; p = 0.352$). We also did not observe any statistically significant associations between leptin and adiponectin levels or leptin/adiponectin, leptin/BMI and adiponectin/BMI ratios and the presence of ILD or PAH or decreased DLCO (Table 6).

Regarding clinical complication of SSc microangiopathy, we did not find any association between analyzed serum adipocytokine levels and the presence of active digital ulcers or nailfold videocapillaroscopy characteristics (data not shown).

A comparison made among patients with SSc stratified according to the activity of the disease showed leptin levels to be significantly lower in active patients than in those with inactive disease ($Z = -2.604; p = 0.009$). Using Spearman’s rank correlation test, leptin serum levels showed a strong negative correlation with score of disease activity index ($r = -0.342; p = 0.018$). Importantly, leptin/BMI ratio was also significantly lower in active patients (420.18 ±502.73) then in those with inactive disease (1410.84 ±1249.33; $p = 0.0032$) and inversely correlated with the value of the activity index ($r = -0.370; p = 0.010$). Activity index score also negatively correlated with leptin/adiponectin ratio ($r = -0.303; p = 0.037$) (Tables 5 and 6).
| Parameter                        | Clinical parameter | Z-value | P-value |
|---------------------------------|--------------------|---------|---------|
| **Active disease (n = 9)**      | Inactive disease (n = 39) |         |         |
| Leptin [pg/ml]                  | 13700.49 ±18293.32 | 39399.28 ±38933.9 | –2.604 | 0.009 |
| Adiponectin [ng/ml]             | 8898.05 ±7925.16   | 12560.27 ±9033.85 | 1.571  | 0.116 |
| Leptin/adiponectin             | 2.80 ±3.31         | 6.13 ±8.41      | 1.556  | 0.119 |
| Leptin/BMI                      | 420.18 ±502.73     | 1410.84 ±1240.33 | –2.943 | 0.0032 |
| Adiponectin/BMI                 | 335.49 ±337.27     | 507.98 ±420.90  | 1.545  | 0.122 |
| **ILD positive (n = 43)**       | ILD negative (n = 5) |         |         |
| Leptin [pg/ml]                  | 36171.35 ±39340.75 | 24544.53 ±10647.40 | –0.155 | 0.876 |
| Adiponectin [ng/ml]             | 12264.97 ±9136.56  | 9240.90 ±6370.34 | –0.343 | 0.731 |
| Leptin/adiponectin             | 5.69 ±8.25         | 4.31 ±3.61      | 0.136  | 0.891 |
| Leptin/BMI                      | 1270.60 ±1277.50   | 941.7784 ±310.22 | 0.077  | 0.937 |
| Adiponectin/BMI                 | 489.45 ±422.92     | 385.47 ±289.96  | 0.381  | 0.703 |
| **Decreased DLCO (n = 9)**      | DLCO in normal range (n = 17) |         |         |
| Leptin [pg/ml]                  | 23815.10 ±18750.11 | 37500.57 ±14336.66 | 0.416  | 0.676 |
| Adiponectin [ng/ml]             | 8598.92 ±51911.20  | 12592.19 ±7981.97 | 1.991  | 0.046 |
| Leptin/adiponectin             | 9.21 ±10.57        | 4.7 ±7.59      | –1.022 | 0.306 |
| Leptin/BMI                      | 890.79 ±754.87     | 1311.29 ±1319.08 | 0.568  | 0.569 |
| Adiponectin/BMI                 | 318.33 ±443.35     | 493.06 ±349.35  | 2.065  | 0.038 |
| **Decreased FVC (n = 6)**       | FVC in normal range (n = 35) |         |         |
| Leptin [pg/ml]                  | 21906.26 ±13823.76 | 41698.69 ±44088.4 | 1.115  | 0.264 |
| Adiponectin [ng/ml]             | 6815.72 ±4990.39   | 14102.66 ±9494.23 | 2.351  | 0.018 |
| Leptin/adiponectin             | 6.84 ±9.12         | 5.39 ±8.22      | –0.283 | 0.777 |
| Leptin/BMI                      | 831.22 ±712.32     | 1436.92 ±1404.22 | 1.239  | 0.215 |
| Adiponectin/BMI                 | 269.14 ±210.61     | 563.21 ±450.10  | 2.111  | 0.034 |
| **PAH positive (n = 11)**       | PAH negative (n = 37) |         |         |
| Leptin [pg/ml]                  | 26867.08 ±22572.98 | 37361.81 ±41390.21 | 0.437  | 0.661 |
| Adiponectin [ng/ml]             | 13066.65 ±10342.41 | 11479.82 ±8635.04 | –0.301 | 0.763 |
| Leptin/adiponectin             | 4.09 ±4.30         | 6.09 ±8.76      | 0.360  | 0.718 |
| Leptin/BMI                      | 987.94 ±818.72     | 1314.55 ±1327.62 | 0.579  | 0.562 |
| Adiponectin/BMI                 | 532.81 ±451.38     | 458.79 ±406.22  | –0.276 | 0.782 |
| **C3 low level (n = 11)**       | C3 in normal range (n = 37) |         |         |
| Leptin [pg/ml]                  | 12026.28 ±12734.57 | 40394.01 ±39711.47 | 2.880  | 0.004 |
| Adiponectin [ng/ml]             | 14512.23 ±13000.93 | 11023.30 ±7715.43 | –0.351 | 0.725 |
| Leptin/adiponectin             | 2.00 ±2.71         | 6.56 ±8.63      | 2.117  | 0.034 |
| Leptin/BMI                      | 488.85 ±567.18     | 1408.71 ±1261.01 | 2.796  | 0.005 |
| Adiponectin/BMI                 | 429.29 ±337.22     | 623.12 ±596.72  | –0.502 | 0.615 |
Importantly, we found no significant difference between the active and inactive patients relative to mean BMI values (28.59 ± 4.24 kg/m² vs. 26.10 ± 4.38 kg/m²; \( p = 0.128 \)). Moreover, there was no statistically significant difference between the active and inactive patients in terms of serum inflammatory markers, ESR (28.22 ± 13.91 vs. 24.33 ± 16.00 mm/h; \( p = 0.267 \)) or CRP (15.31 ± 27.96 vs. 6.24 ± 13.61 mm/h; \( p = 0.405 \)). Similarly, the mRSS score did not differ in patients with active SSc (13.22 ± 7.39) compared to those with inactive disease (9.25 ± 5.77; \( p = 0.165 \)). Although C3 levels were lower in active patients, the difference was not statistically significant (\( p = 0.09 \)) (Table 7).

**Discussion**

In recent years, there are more and more data on the role of adipocytokines in autoimmune responses. Particularly, the role of leptin as a potent pro-inflammatory agent has been investigated in spontaneous models of autoimmunity and studies in humans have further delineated the role of leptin in the pathogenesis of autoimmune diseases such as diabetes, inflammatory bowel disease, Behçet’s disease, systemic lupus erythematosus (SLE), and rheumatoid arthritis (RA) [3–5, 14, 18, 48–54]. Adiponectin, another active hormone released by adipocytes, seems to be an attractive opposite in the field of autoimmune diseases, since it is considered to act as an anti-inflammatory molecule, and recently some studies demonstrated that plasma levels of adiponectin in patients with autoimmune diseases might be related to inflammatory status in their pathogenesis [2, 6, 52, 55–57].

Although several studies have explored the role of circulating leptin and adiponectin levels in SSc patients, they gave inconclusive or contradictory results, and there are only rare reports that have analyzed both adipocytokines concomitantly.

Our study sought to compare serum leptin and adiponectin levels in SSc patients according to healthy controls and to determine whether leptin and adiponectin are associated with clinical profiles of the patients. Since leptin and adiponectin seem to have an opposing action in inflammatory and immune responses, we aimed to assess whether their abnormal ratio (leptin/adiponectin; L/A) rather than isolated values might be associated with disease manifestations. Moreover, since many factors, including gender, age and BMI, could influence adiponectin and leptin levels in serum, all patients and subjects from the control group were females adjusted for age, and did not differ significantly in BMI or lipid profile, except for higher triglycerides (TG) values among SSc patients. The analyzed laboratory data were adjusted to these demographic parameters to facilitate comparison of the evaluated groups (patients and controls).

### Table 6. Cont. Comparison of serum adipocytokine levels and ratios among SSc patients according to pulmonary involvement, disease activity and C3 or C4 complement concentration (Mann-Whitney U-test)

| Parameter          | Clinical parameter | Z    | P-value |
|--------------------|--------------------|------|---------|
|                   | C4 low level (n = 4) |      |         |
| Leptin [pg/ml]     | 14910.16 ± 19968.36 | 36066.83 ± 38293.32 | 1.481 | 0.138 |
| Adiponectin [ng/ml]| 19582.07 ± 15559.86 | 11119.65 ± 8033.81 | –1.029 | 0.303 |
| Leptin/adiponectin | 2.93 ± 15.12       | 5.82 ± 8.15       | 1.305 | 0.191 |
| Leptin/BMI         | 581.44 ± 805.26     | 1268.48 ± 1224.77 | 1.481 | 0.138 |
| Adiponectin/BMI    | 884.72 ± 829.69     | 436.51 ± 347.00   | –0.991 | 0.321 |

BMI – body mass index, C3 – C3 complement concentration, C4 – C4 complement concentration, DLCO – diffusing capacity of the lungs for carbon monoxide, TLC – total lung capacity, ILD – interstitial lung disease, PAH – pulmonary arterial hypertension, FVC – forced vital capacity. Results are given as mean ± SD.

### Table 7. Comparison of important inflammatory and clinical markers between patients with active and inactive disease (Mann-Whitney U test)

| Parameter | Active disease | Inactive disease | Z     | P-value |
|-----------|---------------|-----------------|-------|---------|
| ESR [mm/h]  | 28.22 ± 13.91 | 24.33 ± 16.00   | –1.109 | 0.267   |
| CRP [mg/ml] | 15.31 ± 27.96 | 6.24 ± 13.61    | –0.832 | 0.405   |
| C3         | 0.99 ± 0.24   | 1.12 ± 0.19     | 1.675  | 0.09    |
| C4         | 0.22 ± 0.08   | 0.24 ± 0.08     | 0.969  | 0.332   |
| mRSS       | 13.22 ± 7.39  | 9.25 ± 5.77     | –1.386 | 0.165   |
| BMI        | 28.59 ± 4.24  | 26.10 ± 4.38    | –1.518 | 0.128   |

ESR – erythrocyte sedimentation rate, CRP – C-reactive protein, C3 – C3 complement concentration, C4 – C4 complement concentration, mRSS – modified Rodnan skin score, BMI – body mass index. Results are given as mean ± SD.
In our study, there was no significant difference of leptin and adiponectin levels in sera of SSc patients as compared to healthy controls or of leptin/adiponectin ratio between these groups; however, in SSc patients only a strong negative correlation was observed between leptin and adiponectin serum levels. This is in agreement with two recent meta-analyses – one of fourteen papers (six for serum leptin, six for serum adiponectin and two for both) performed by Zhao et al. [26] and the second of eleven studies (51 SSc patients and 341 controls) by Lee et al. [31] that showed no significant differences in serum leptin levels between SSc patients and healthy controls. However, in contrast to our observation, both meta-analyses revealed significantly lower serum adiponectin levels in SSc patients than in normal controls, but neither the leptin/adiponectin ratio nor a correlation between leptin and adiponectin levels was reported by those authors. When considering respective literature data concerning either serum leptin or adiponectin levels in SSc, they are inconsistent. Our results are in agreement with data from two previous studies by Olewicz-Gawlik et al. [32] and Budulgan et al. [33], who observed only slightly lower leptin levels in SSc patients than in healthy subjects, though not down to significant levels, and found no statistically significant differences in serum adiponectin. Similar to our report, serum adiponectin levels were also comparable between the SSc group and controls in the studies of other authors [34–36]. However, Kotulska et al. [37] as well as Winsz-Szczotka et al. [38, 39] reported a significantly lower serum leptin level in SSc patients, whereas the results of two recent studies detected significantly higher levels of circulating leptin in SSc subjects with respect to healthy controls [2, 40]. Although the present studies did not compare adiponectin and leptin levels in SSc with those in other autoimmune diseases, studies in RA and SLE have shown that these disorders are generally associated with elevated serum adiponectin or leptin [58–60]. Thus, our observations might indicate that, in contrast, SSc is not associated with abnormal levels of measured adipocytokines, despite the chronic inflammation that is thought to be a hallmark of this disease [36, 61].

These discrepancies between particular studies might result from the heterogeneity of SSc patients according to demographic parameters such as age, gender, BMI as well as the clinical profile of the disease or immunosuppressive treatment used.

Body mass index is one of the most potent factors influencing serum adipocytokine levels. In fact, in our study there was a strong positive correlation between either leptin levels or leptin/adiponectin ratio and BMI but only among SSc patients and, in contrast, adiponectin concentration was strongly inversely correlated with BMI in the patient group but not in healthy controls. Similarly, Kotulska et al. [37] reported a significant positive correlation of serum leptin with BMI only in SSc patients. This is in agreement with the notion that a relationship between BMI and leptin levels is not observed for normal individuals [62]. The authors indicated that a decreased serum leptin level and BMI may be a consequence of gastrointestinal tract involvement and low energy status with decreased fat stores and fasting in SSc patients since the lowest leptin levels were found in patients with more advanced disease [37]. In fact, in the study of Budulgan et al. [33], BMI values as well as leptin levels were significantly lower in the patient group than in controls and, similarly, Toussirot et al. [63] and Sari et al. [64] noted a possible relationship between reduced leptin levels and low BMI values. In patients with SSc, fat mass distribution may be different to that in controls. In particular, patients with SSc display lower subcutaneous adipose tissue accumulation than controls despite having similar BMI, as was also observed in RA patients [65]. This is attributed to skin fibrosis, and attrition of dermal white adipose tissue (dWAT) has been reported to correlate with reduced levels of circulating adiponectin in SSc patients [66]. This is in favor of our results; however, we did not evaluate fat stores or the loss of dWAT in our patients.

Moreover, disease duration has been reported to have an effect on serum adiponectin and leptin levels [67]. In fact, we observed a positive, close to significant correlation between serum adiponectin and the years from first non-Raynaud manifestation of the disease, and this correlation reached statistical significance when we analyzed adiponectin/BMI ratio. In favor of these results is the observation reported by Lakota et al. [36], who found low adiponectin levels in patients with disease in a relatively early stage (arbitrarily defined as fewer than 18 months from the first non-Raynaud manifestation) in comparison with patients with disease in the late stage (defined as more than 36 months). In contrast, the levels of adiponectin did not correlate with disease duration of investigated patients in the study by Olewicz-Gawlik et al. [32]. On the other hand, we did not find a significant correlation of leptin, L/A ratio and leptin/BMI according to disease duration, which is in agreement with data from previous studies [2, 37, 40]. In contrast, Winsz-Szczotka et al. as well as Olewicz-Gawlik et al. [32] reported a significant positive correlation between leptin and disease duration [38], but an inverse correlation of both leptin and adiponectin with the duration of the first non-RP symptom has also been reported [39]. Such conflicting results might be a consequence of different disease progression rate and severity in the studied group of SSc patients.

In fact, additionally to the role in inflammation, opposite action of leptin and adiponectin has been described in the field of connective tissue remodeling and fibrosis. Leptin seems to be a profibrogenic molecule, whereas adiponectin has been found to have antifibrogenic action [66, 68–72]. Profibrotic effects of leptin have been...
reported in bleomycin-induced lung fibrosis in mice by augmentation of transforming growth factor-β (TGF-β) signaling, and in liver fibrosis, leptin acts as an activator of hepatic stellate cells (HSCs) and stimulates the transcriptional activation of collagen I and tissue inhibitor of metalloproteinases 1 (TIMP1) [71, 73, 74]. In contrast, adiponectin might inhibit the collagen gene expression and myofibroblast differentiation [70].

The most distinctive feature of tissue fibrosis in SSc is skin thickening and pulmonary interstitial fibrosis [61]. Significantly, recent studies demonstrated that adiponectin levels are reduced in patients with diffuse skin involvement and correlate with disease severity [36, 41]. In general, our findings are in agreement with these suggestions. When considering disease subtype, diffuse form (dcSSc) was associated with significantly lower serum adiponectin levels and a close to significant decrease in adiponectin/BMI ratio compared to patients with limited subtype (lcSSc). Moreover, stratification by disease subtype revealed that both adiponectin and adiponectin/BMI ratio were significantly lower in the diffuse SSc group, but not the limited SSc group, than in controls. Although the results indicate a weak association, and should be treated with caution, this finding is in harmony with antifibrotic functions of this adipocytokine, and they are concordant with the data of recent studies.

In particular, this is in agreement with results from the meta-analysis by Lee et al. [31] which revealed that the adiponectin level was significantly lower in the diffuse SSc group, but not the limited SSc group, than in controls. This is also in line with the observation of Masiu et al. [34] and Lakota et al. [36] as well as Arakawa et al. [41]. In addition, in the study of Arakawa et al. [41], adiponectin mRNA levels in skin tissues from patients with dcSSc were also reduced. On the other hand, Olewicz-Gawlik et al. [32] did not observe statistically significant differences in adiponectin serum concentrations between dcSSc and lcSSc subgroups.

The difference in adiponectin levels between patients with lcSSc and those with dcSSc suggests that adiponectin might be associated with the severity of skin fibrosis. However, we did not find any correlation between either leptin or adiponectin and the value of total skin thickness score assessed by mRSS. Similarly, Pehliván et al. and Budulgan et al. [2, 33] found no association between leptin levels and skin score in the SSc patients, but in the earlier studies, a negative correlation was observed, despite similar adiponectin serum levels between patients with SSc and healthy controls [35, 36, 41]. Moreover, Lakota et al. [36] reported a significant correlation between adiponectin mRNA expression in the skin and mRSS in the individual biopsies. This discrepancy might result from the different proportion of disease subtypes among SSc patients in particular reports, since in our study only 6 from 48 patients had dcSSc, while in those that reported a significant correlation, the ratio of leptin or adiponectin to BMI was relatively high compared to our patients.
Clinical associations of serum leptin and leptin/adiponectin ratio in systemic sclerosis

The inverse correlation between leptin and disease activity as observed in our study might be somewhat intriguing since leptin is assumed to be a strong pro-inflammatory agent increasing the synthesis of pro-inflammatory cytokines such as IL-6, IL-12 and TNF-α [6, 8–11, 13]. In fact, in the literature there are some data on a strong positive correlation between leptin levels and disease activity in other rheumatic diseases, including SLE [4, 49–52, 58], RA [53, 75–77] and ankylosing spondylitis (AS) [64, 78], but to date the clinical significance of this elevation remains unknown. It is thought that serum leptin levels may be either a contributing factor or a marker of disease activity [14, 49, 54]. However, recent studies have suggested that leptin has a dual role in inflammation since it might express certain anti-inflammatory properties by releasing IL1 receptor antagonist (IL-1Ra) and increasing the production of anti-inflammatory IL-4 [13–15, 79, 80]. Furthermore, lower leptin levels in active patients might be explained by the mechanism that in the chronic inflammatory process, leptin is inhibited by long-acting pro-inflammatory cytokines in adipose tissue [17]. In fact, serum leptin level has been shown to have a negative correlation with inflammatory markers such as CRP and IL-6 in patients with RA and prolonged in vitro stimulation of adipose tissue with TNF-α inhibits the production of leptin and leptin mRNA [81]. It may also be possible that leptin release is reduced during the active period and its anti-inflammatory influence is decreased.

For better evaluation of the association between leptin levels and disease activity, we further analyzed particular parameters, including systemic markers of inflammation, that can influence the activity index score. In this study, we found no correlation between either acute phase reactant CRP or ESR and leptin levels and, furthermore, there was no difference between the active and inactive patients with regard to CRP and ESR, which is in agreement with the previous observations of Budulgan et al. [33]. Moreover, lower leptin levels in active patients might be associated with lower BMI; however, in our study, we did not find a significant difference between active and inactive patients in terms of BMI. What is more, active and inactive patients did not differ according to skin involvement. However, we observed a significantly lower C3 complement level in patients with active disease as well as a strong, statistically significant positive correlation between serum leptin levels as well as leptin/BMI ratio and either C3 or C4 complement levels in SSc patients. Similar results concerning the association between leptin levels and C3 have been obtained by other authors [32, 33, 37]. Although adiponectin levels were not related to the concentrations of either C3 or C4 complement concentrations, leptin/adiponectin ratio was decreased in patients with lower C3 status and showed a strong positive correlation with C3.
Since low C3 and C4 status may indicate endothelium activation or damage, our observations might suggest that lower leptin levels and leptin/adiponectin ratio in SSc patients with more active disease reflect vascular homeostasis rather than inflammation. In fact, leptin receptors are expressed on vascular endothelial cells and smooth muscle cells and leptin facilitate proliferation of human endothelial cells (ECs), supporting angiogenesis and neovascularization [82–84]. In contrast to leptin, adiponectin may play a role in the inflammatory vascular response by inhibiting the expression of adhesion molecules such as VCAM-1, ICAM-1 and E-selectins on endothelial cells, preventing activation and migration of immune cells [85]. Although there are no data concerning leptin or adiponectin levels in relation to aspects of microangiopathy in SSc patients, it is noteworthy that lower median plasma levels of adiponectin were found in subjects with diabetic foot as well as a significant negative correlation between adiponectin and diabetic retinopathy, which suggest a possible role of hypoadiponec- 
tinemia in microvascular involvement [86]. Nevertheless, we did not find a statistical association between leptin or adiponectin levels and microvascular complications in our SSc patients. Specifically, neither leptin nor adiponectin showed a significant difference between patients with digital ulcers and those without.

Conclusions

The results of our study indicate that, in contrast to other autoimmune diseases, SSc does not seem to be associated with abnormal levels of leptin or adiponectin, despite the chronic inflammation. However, according to our results, leptin and adiponectin might be differentially expressed in SSc sera and might be associated with different disease manifestations. In particular, we observed that lower adiponectin serum level as well as adiponectin/BMI ratio are associated with more severe skin involvement as well as with restriction on pulmonary function tests, and this finding is in harmony with its antifibrotic action, whereas leptin levels inversely correlated with disease activity index. Decreased serum leptin was associated with greater disease activity, which is somewhat intriguing in the face of its expected strong pro-inflammatory action. In this aspect, since we did not find a correlation between serum leptin and systemic markers of inflammation such as CRP and ESR which can influence the activity index score in SSc patients, it might be supposed that leptin’s association with disease activity is independent of its contribution to inflammatory processes, which is in contrast to other rheumatic diseases. Instead, our study showed that lower serum leptin levels as well as decreased leptin/adiponectin ratio were related to a low status of complement component C3 or C4, thus suggesting that the association of leptin deficiency with higher disease activity reflects vascular status rather than systemic inflammation.

However, it has to be noted that our study has several limitations. The first of them is the small and homogeneus sample. Another potential drawback of the study may be a selection bias. Thus, further research enrolling larger groups of SSc patients as well as comparative studies with other rheumatic diseases such as SLE or RA seem to be justified.

Systemic sclerosis does not seem to be associated with abnormal levels of leptin or adiponectin, despite the chronic inflammation. Leptin and adiponectin are differentially expressed in SSc sera and might be associated with different disease manifestations. Low adiponectin status seems to be associated with intensity of cutaneous and pulmonary repercussions of tissue fibrosis in SSc. Decreased leptin levels might be related to more active disease, in particular reflecting vascular status in SSc patients.

Conflict of interest

The authors declare no conflict of interest.

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