

# CIRCULATING LEPTIN AND RESISTIN LEVELS IN A ROMANIAN RHEUMATOID ARTHRITIS POPULATION

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## Abstract

**Objective.** Rheumatoid arthritis (RA), a chronic autoimmune disease which primarily affects the joints, is associated with various cardiometabolic comorbidities. These comorbidities, particularly metabolic syndrome, exert an altered secretion of proinflammatory adipokines that increase cardiovascular risk in rheumatic diseases. Leptin and resistin are two of the most important and intensely-studied adipocytokines. Increased serum levels of both leptin and resistin in RA patients have been previously reported but the results are conflicting. This study aimed to investigate the serum levels of leptin and resistin in a Romanian rheumatoid arthritis population and to compare them with the serum concentrations of healthy controls.

**Material and methods.** We assessed clinical and biochemical parameters in 84 RA patients and 44 healthy controls with the help of specific enzyme-linked immunosorbent assays.

**Results.** Mean disease duration was 12.1±9.6 years and RA patients had a mean disease activity score of 4.24±1.3, as assessed by Disease Activity Score-28 using C-reactive protein (DAS-28 CRP). RA patients showed significantly higher concentrations of serum leptin than controls (median 28.63 ng/ml vs. 21.16 ng/ml, p=.03) while there was a trend for an elevated serum resistin level in RA patients (median 15.98 ng/ml vs. 14.05 ng/ml) which did not reach statistical significance (p = 0.41).

**Conclusions.** The current study shows a significant increase in circulating leptin but not resistin in RA patients than in controls. The link between adipokines, the chronic pro-inflammatory state and consequent cardiometabolic complications warrants further larger and longitudinally-designed studies.

**Keywords:** leptin, resistin, adipokines, rheumatoid arthritis, cardiometabolic risk

## INTRODUCTION

Rheumatoid arthritis (RA) is a chronic autoimmune disease which primarily affects the joints but is also associated with various systemic comorbidities (1). Among them, the cardiometabolic involvement is significantly regarded responsible for the increased morbidity and mortality rates encountered in these populations (2). Traditional risk factors cannot entirely explain the pathogenesis of accelerated

atherosclerosis in RA, chronic inflammation playing a key role in this process (3).

RA has been linked to various metabolic changes, such as altered body composition (increased visceral fat mass and decreased lean mass, resulting in little or no change in body-mass index, the so-called "rheumatoid cachexia"), insulin resistance and peculiar patterns of serum lipids (4). Patients with RA also have a higher prevalence of metabolic syndrome

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(MetS), as reported in a meta-analysis by Zhang et al. (5).

In the last decades, significant progress has been made in the understanding of the complex relationships between adipose tissue, inflammation and the immune functions. Besides its role of energy storage, white adipose tissue (WAT) is now considered an active endocrine organ, secreting adipokines and thus regulating systemic metabolism. These bioactive peptides have been shown to modulate both physiological and pathological processes, such as energy homeostasis and metabolic dysfunctions (6). They could represent the link between inflammation and cardiometabolic comorbidities (7). Thus MetS, especially through the altered secretion of proinflammatory adipokines, may exert a pivotal role in the increased cardiovascular risk in rheumatic diseases.

Same adipokines have been reported with both pro- and anti-inflammatory effects, leading to the idea that their expression is influenced by the surrounding milieu and disease state (8). Therefore, these pleiotropic molecules could enhance the proinflammatory state and concomitant atherosclerosis in RA patients (7). The relationship seems bidirectional, with adipokines being also considered as potential diagnostic biomarkers for various rheumatic diseases, such as RA (9) and systemic lupus erythematosus (10).

Leptin and resistin are two of the most important and intensely-studied adipocytokines. Leptin was the first discovered adipokine in 1994 and plays a key role in appetite and body weight homeostasis, by promoting satiety and increasing energy consumption (11). It has also been reported to modulate various processes such as bone metabolism, vascular function, inflammation and immune responses, exerting proinflammatory activities through cytokine induction, chemotaxis, macrophage activation and naïve T-cell proliferation (12). Resistin is an adipokine mainly produced by macrophages, with some contribution from adipocytes (13). It has been shown to promote insulin resistance and may also contribute to the endothelial dysfunction and to the disturbed immune response (14). Furthermore increased serum levels of both leptin and resistin in RA patients have been previously reported but the results are conflicting (15). Therefore, the aim of this study was to assess serum concentrations of leptin and resistin in a Romanian rheumatoid arthritis population and to compare them with healthy controls.

## MATERIAL AND METHODS

### Patient selection

84 RA patients, diagnosed on the basis of the 2010 American College of Rheumatology (ACR)/European League against Rheumatism (EULAR) classification criteria (16), were recruited in the Rheumatology Department from the “Iuliu Hatieganu” University of Medicine and Pharmacy, Cluj-Napoca, Romania. The exclusion criteria were represented by age under 18, weight loss diet in the last 30 days, weight loss treatment in the last 90 days, diabetes mellitus, any history of atherosclerotic CV disease (including documented coronary heart disease, stroke and peripheral arterial disease), malignancy and infections during the last 30 days. 44 subjects age-sex matched from the outpatient service of the 2<sup>nd</sup> Internal Medicine Department of the same University represented the control group. The study was conducted in accordance with the Declaration of Helsinki and was approved by the institutional Ethics Committee of the University. Written informed consent was obtained prior to enrollment from all participants.

### Clinical assessment

We performed a thorough clinical evaluation and recorded various parameters such as age, disease duration, body weight and height, waist circumference, smoking status and current medication. The diagnosis of metabolic syndrome was based on the International Diabetes Federation criteria (17) and that of arterial hypertension according to current European guidelines (18). Disease activity of RA patients was assessed with the Disease Activity Score-28 using CRP (DAS28-CRP) (19) and functional status was determined by the Health Assessment Questionnaire (HAQ) (20).

### Laboratory analysis

Fasting serum creatinine, glucose, uric acid, total cholesterol, LDL-cholesterol, high-density lipoprotein cholesterol and triglycerides were determined in the hospital clinical laboratory (Cobas Mira Plus analyzer, Hoffman La Roche, Switzerland). C-reactive protein (CRP) and IgM rheumatoid factor (RF, negative < 32 UI/ml) were assessed by automated turbidimetry, whereas anti-cyclic citrullinated peptide antibodies (anti-CCP, negative < 5 UI/ml) by enzyme-linked immunosorbent assay (ELISA). For

leptin and resistin ELISA determinations serum was obtained after centrifugation and kept frozen at  $-70^{\circ}\text{C}$  until use. The levels of these adipokines were determined using DuoSet Human ELISA kits (R&D System, Minneapolis, USA) according to the manufacturer's instructions, with minimum detectable concentrations of 0.31 ng/ml.

### Statistical analysis

Statistical analysis was performed using the MedCalc Statistical Software version 19.2.1 (MedCalc Software Ltd, Ostend, Belgium; <https://www.medcalc.org>; 2020). Continuous data were tested for normality of distribution with Shapiro Wilk test and was expressed by mean  $\pm$  standard deviation or median and 25-75 percentiles. Qualitative variables were characterized by frequency and percentage. Comparisons between groups were carried out with Student t test, Mann-Whitney test or chi-square test, whenever appropriate. A p value  $< 0.05$  was considered statistically significant.

## RESULTS

Baseline characteristics of the RA patients and control groups are displayed in Table 1. There were no statistically significant differences between the two groups in terms of clinical, medical history and basic laboratory values.

RA patients exhibited mean disease duration of  $12.1 \pm 9.6$  years. 61 (72.61%) of them were RF posi-

tive while 66 (78.6%) were anti-CCP positive with a mean DAS28-CRP score of  $4.24 \pm 1.3$ . Regarding current antirheumatic agent use, 22 (26.2%) were taking prednisone, 69 (82.1%) were on synthetic disease-modifying anti-rheumatic drugs and 28 (33.3%) were on biologic disease-modifying anti-rheumatic drugs.

RA patients showed significantly higher concentrations of serum leptin than controls (median 28.63 ng/ml vs. 21.16 ng/ml,  $p = 0.03$ ) while there was a trend for an elevated serum resistin level in RA patients (median 15.98 ng/ml vs. 14.05 ng/ml) which did not reach statistical significance ( $p = 0.41$ ). There were no significant correlations between adipokines and RA clinical or laboratory parameters.

## DISCUSSIONS

The present study reports that leptin levels were higher in the Romanian RA population than in controls, with no statistically significant differences in resistin concentrations between the two groups. Our results are in line with a previous study by Otero et al. showing markedly higher serum leptin levels, along with other studied adipokines (adiponectin and visfatin), whereas resistin levels were similar to those from controls (21). Furthermore, a meta-analysis from 2014 performed by Tian et al. on 20 studies including 998 RA patients and 692 controls concluded that leptin levels were higher in RA patients than controls (22). Another 2016 meta-analysis also

**TABLE 1.** Characteristics of the two study groups

Parameter	RA patients (n = 84)	Controls (n = 44)	p value
Age, years	54.8 $\pm$ 11.7	51.4 $\pm$ 12	.06
Women, n (%)	71 (84.5)	37 (84.1)	.1
BMI, kg/m <sup>2</sup>	27.4 $\pm$ 5.4	25.9 $\pm$ 3.8	.1
WC, cm	92.5 $\pm$ 12.9	90.2 $\pm$ 11	.31
TC, mg/dl	204.2 $\pm$ 45.6	210.2 $\pm$ 52.9	.51
HDL-C, mg/dl	55 (48;63)	54 (44;60)	.37
TG, mg/dl	105 (73;139)	114 (68;181)	.37
LDL-C, mg/dl	127.6 $\pm$ 39	131.4 $\pm$ 53.5	.68
Hgb, g/dl	12.8 (12.1;13.7)	13.3 (13.2;14.4)	.11
MetS, n (%)	19 (22.6)	12 (27.3)	.71
Dyslipidemia, n (%)	42 (50)	24 (54.5)	.76
Hypertension, n (%)	31 (36.9)	10 (22.7)	.15
Current smoking, n (%)	14 (16.7)	11 (25)	.28
<b>Adipokines</b>			
Leptin, ng/ml	28.63 (12.59;46.21)	21.16 (8.11;33.61)	<b>.03</b>
Resistin, ng/ml	15.98 (9.58;25.81)	14.05 (10.93;20.61)	.41

\*BMI= body mass index; WC= waist circumference; TC= total cholesterol; HDL-C= high density lipoprotein cholesterol; TG= triglycerides; LDL-C= low density lipoprotein cholesterol; Hgb= hemoglobin; MetS= metabolic syndrome; Values are presented as median (Quartile 1; Quartile 3) and mean  $\pm$  SD, respectively

demonstrated that the circulating leptin levels were significantly higher in patients with RA reporting also a small significantly positive correlation between leptin levels and RA activity (23). Conversely, Popa et al. found no significantly elevated plasma leptin and reported an inverse correlation between inflammation and leptin concentrations, suggesting that active chronic inflammation may lower plasma leptin levels (24). Reasons for these disparities may be represented by the small sample sizes, clinical heterogeneity (including disease duration, current disease activity state) and/or medication use.

The other adipokine studied, resistin, was higher in our RA group than in controls but without reaching statistical significance. In support of the present results, a study by Yoshino et al. revealed no statistically significant differences in serum resistin levels between the RA patients and normal controls, while the serum leptin concentrations were significantly higher in the former group (25). However, Migita et al. found significantly elevated serum resistin levels in 42 patients with RA compared with 38 healthy controls, suggesting that resistin may exert an important role in the inflammatory process (26). Another study compared serum and synovial resistin levels from patients with RA and osteoarthritis and reported higher concentrations in the former group (27). Sources of the heterogeneity of the results may be the same as mentioned for the leptin studies, along with various ELISA assays.

The present study has several limitations including a relatively small sample size and the cross-sectional design, which precludes drawing inferences on the direction of causality. The heterogeneity of treatment is another factor that should be taken into

account when interpreting the results. Additionally, we could not determine whether other concomitant diseases (e.g. MetS or hypertension) could have influenced the relationship between rheumatoid arthritis and adipokines.

## CONCLUSIONS

Our study shows a statistically significant increase in circulating leptin but not resistin in RA patients than in controls. The link between adipokines (along with other cytokines released by WAT), the chronic pro-inflammatory state and consequent cardiometabolic complications warrants further larger and longitudinally-designed studies. Understanding the exact actions and mechanisms of adipokines would be of paramount importance as it could help to more effectively classify their inherent cardiometabolic risk and could thus provide novel therapies for autoimmune diseases.

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This study was performed in line with the principles of the Declaration of Helsinki. Approval was granted by the Ethics Committee of the “Iuliu Hatieganu” University of Medicine and Pharmacy (Date 02.06.2015/No. 319). Informed consent from individuals to publish their data in an anonymized form was obtained. All study data are available upon request from the authors.

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