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Leptin and inflammation in patients with chronic heart failure

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Abstract

Background: There is an increasing interest in the role of leptin in cardiovascular pathophysiology, including proinflammatory effects. Many studies have reported elevated leptin levels in non-cachectic patients with chronic heart failure (CHF), however, the role of leptin in CHF remains unclear.

Aim: To assess the relationship between leptin levels in patients with CHF and left ventricular (LV) systolic dysfunction in relation to ventilatory response to exercise and hsCRP levels.

Methods: The study group consisted of 41 patients (mean age 50.2 ± 9.3 years) with stable CHF and LV ejection fraction < 45% and eight healthy controls (mean age 43.6 ± 14.7 years). Sixteen (39%) patients had coronary artery disease. All subjects underwent anthropometric measurements (weight, height, and waist circumference), standard echocardiography, and maximal cardiopulmonary exercise treadmill test. Biochemical analysis included the assessment of leptin and hsCRP levels as well as white blood count (WBC) and erythrocyte sedimention rate.

Results: Leptin levels, including body mass index (BMI)-adjusted leptin levels, were significantly higher in the CHF patients than in the controls (9.2 ± 7.5 *vs* 2.9 ± 1.25 ng/mL; $p = 0.005$). We found significantly higher WBC, neutrophil count, lymphocyte percentage and BNP levels in the CHF group *vs* controls. There were significant correlations in the CHF group between leptin levels and BMI ($r = 0.55$; $p < 0.05$), waist circumference ($r = 0.49$; $p < 0.05$), leukocyte count ($r = 0.41$; $p < 0.05$), hsCRP levels ($r = 0.34$; $p < 0.05$), and peak VO_2 ($r = –0.34$; $p < 0.05$). Multivariate step forward regression analysis showed that peak VO_2 was significantly related with leptin levels. After adding VE/VCO_2 slope to the multivariate regression analysis model, only VE/VCO_2 slope was independently associated with leptin levels.

Conclusions: There is a significant relationship between serum leptin levels and peak VO_2, VE/VCO_2 slope and levels of inflammatory markers in patients with CHF.

Key words: chronic heart failure, leptin, C-reactive protein, inflammation, cardiopulmonary exercise test, VO_2

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INTRODUCTION

There is growing interest in the role of leptin in cardiovascular pathophysiology [1, 2]. Many studies have reported elevated levels of leptin in non-cachectic patients with chronic heart failure (CHF) [3, 4] and low levels in cachexia [5], however its role in the pathophysiology of CHF remains unclear. Hyperleptinaemia correlates with serum levels of TNF-α and insulin; therefore it has been suggested that leptin may play a role in an impaired regulation of energy metabolism in CHF [4, 6]. Other suggestions have included that it plays a proinflammatory role [7]. We analysed leptin levels in patients with CHF and systolic left ventricular (LV) dysfunction in relation to peak VO_2 and C-reactive protein (CRP) levels.

METHODS

Patients

The study group consisted of 41 consecutive patients with CHF and LV ejection fraction (LVEF) < 45% (assessed by

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Table 1. Characteristics of patients with chronic heart failure (CHF) and controls

	CHF (n = 41)	Controls (n = 8)	P
Age (years)	50.1 ± 9.3	43.6 ± 14.7	NS
Male/female (%)	33/8 (80/20%)	6/2 (75/25%)	NS
Coronary artery disease	16 (39%)	–	–
Diabetes mellitus	8 (19.5%)	–	–
Left ventricular ejection fraction [%]	26.1 ± 8.1	–	–
New York Heart Association class	2.5 ± 0.8	–	–
Body mass index [kg/m^2]	28.2 ± 4.5	23.7 ± 2.0	0.008
Waist circumference [cm]	99.8 ± 13.3	86.0 ± 7.1	0.007

echocardiography) referred for cardiopulmonary exercise testing. Coronary artery disease was diagnosed in 16 (39%) patients and dilated cardiomyopathy in the remaining 25 (61%). At the time of examination, all patients were in a stable clinical condition and receiving optimal medical therapy. Exclusion criteria were: acute or chronic inflammatory condition; recent myocardial infarction or revascularisation (≤ 3 months); exertional angina or arrhythmias; atrial fibrillation; severe pulmonary disease; and severe renal insufficiency or other organ disorders significantly compromising subjects' physical capacity. Thirty-six (87%) patients were treated with angiotensin-converting enzyme inhibitors or angiotensin-receptor blockers, 38 (93%) with β-blockers, 35 (85%) received furosemide, 26 (63%) were on aldosterone antagonist, ten (24%) were on digoxin, 24 (58%) on aspirin, and ten (24%) were treated with antiarrhythmic drugs. The control group consisted of eight healthy volunteers of a similar age and gender distribution to the patients with CHF.

Cardiopulmonary exercise test

All enrolled subjects underwent a maximal cardiopulmonary exercise treadmill test performed according to the modified Bruce's protocol (adding stage 0: 3 min, 1.7 km/h, 5% grading). The peak oxygen consumption (peak VO_2), carbon dioxide production (VCO_2), and minute ventilation (VE) were measured via the breath by breath technique, using Sensor Medics, model Vmax29. The equipment was calibrated before each test. Patients were encouraged to continue to the limit allowed by their symptoms by the supervising physician. There was continuous ECG monitoring and blood pressure was measured at each stage of exercise. Peak VO_2 was defined as the highest 20-s average during the last 60 s of exercise. The predicted VO_2% was calculated using the Wasserman's equation [8]. Respiratory exchange ratio > 1.0 was taken to represent an adequate effort. The VE/VCO_2 slope was calculated from the whole exercise period. The ventilatory anaerobic threshold was determined by the V-slope method.

Laboratory measurement

Venous blood was drawn before the exercise test after at least 20 min of rest in a supine position. Serum leptin levels were measured with the RIA test. Brain natriuretic peptide (BNP) levels were measured using the Abbott AxSYM Immunoassay system. All subjects gave their informed consent to participate in the study protocol that had been earlier approved by the local Ethics Committee.

Statistical analysis

The results are presented as means and standard deviations or numbers and percentages. The t-Student, Mann-Whitney and χ^2 tests were used to evaluate the significance of differences between the analysed variables. Correlations between the variables were assessed using the Spearman's rank test. Multivariate regression analysis was used to assess which variables showed a significant correlation with leptin and which were independently related with leptin levels. A p value < 0.05 was regarded significant. All analyses were performed using the Statistica 7.0 package.

RESULTS

Clinical characteristics of both groups are shown in Table 1. The subjects with CHF had higher body mass index (BMI) and waist circumference in comparison to the controls. The peak VO_2 was 17.0 ± 5.1 mL/kg/min and VE/VCO_2 slope was 35.3 ± 7.5 in the whole study group. Peak VO_2 was lower in the patients than in controls (17.0 ± 5.1 *vs* 36.9 ± ± 4.9 mL/kg/min, respectively; p = 0.00000).

Results of the laboratory assessments are shown in Table 2. Leptin levels (and leptin levels corrected for BMI) were significantly higher in the CHF patients than in controls. There were significant differences between patients and controls in BNP levels, leukocyte and neutrophil counts, and trend in percentage of lymphocytes.

Ten patients had BMI < 25 kg/m^2. There were significant differences between these patients and controls in leptin levels (respectively: 7.5 ± 10.2 *vs* 2.9 ± 1.2 ng/mL; p = = 0.004) and leptin/BMI (0.34 ± 0.5 *vs* 0.12 ± 0.05; p = 0.003,

Table 2. Results of laboratory assessments in patients with chronic heart failure (CHF) and controls

	CHF (n = 41)	Controls (n = 8)	P
Leptin [ng/mL]	9.2 ± 7.5	2.9 ± 1.2	0.005
Leptin/body mass index	0.32 ± 0.3	0.13 ± 0.05	0.009
Brain natriuretic peptide [pg/mL]	350 ± 520	14 ± 19	0.0005
Haemoglobin [mmol/L]	9.0 ± 0.8	8.6 ± 0.6	NS
Leukocyte count (1 × 10^3)	7.4 ± 2.25	4.9 ± 0.65	0.0008
Neutrophils count (1 × 10^3)	4.37 ± 1.3	2.85 ± 0.4	0.0009
Neutrophils [%]	61.8 ± 8.2	57.8 ± 5.2	NS
Lymphocytes [%]	26.3 ± 8.1	31.2 ± 6.5	0.07
Erythrocyte sedimentation rate [mm/h]	10.2 ± 10.1	5.9 ± 3.1	NS
High-sensitivity C-reactive protein [mg/L]	5.74 ± 8.1	0.75 ± 0.74	NS
Creatinine [mmol/L]	103.5 ± 29.9	–	–
Total cholesterol [mmol/L]	5.2 ± 1.3	–	–
High-density lipoprotein cholesterol [mmol/L]	1.26 ± 0.9	–	–
Low-density lipoprotein cholesterol [mmol/L]	3.19 ± 1.0	–	–
Triglycerides [mmol/L]	2.2 ± 1.3	–	–
Glucose [mmol/L]	6.3 ± 1.3	–	–

respectively), despite no significant differences in BMI (22.7 ± 1.7 *vs* 23.7 ± 2.0, NS) or waist circumference (85.3 ± ± 10.5 *vs* 86.0 ± 7.1 cm, NS).

Eight of the CHF patients had diabetes mellitus. There were no significant differences between the diabetics and the rest of the CHF group in terms of leptin levels or leptin/BMI.

There were significant correlations in the CHF group between leptin levels and BMI ($r = 0.55$; $p = 0.0002$), waist circumference ($r = 0.49$; $p = 0.001$), leukocyte count ($r = = 0.41$; $p = 0.008$), hsCRP levels ($r = 0.39$; $p = 0.01$), peak VO_2 ($r = –0.34$; $p = 0.03$) and VE/VCO_2 slope ($r = 0.33$; $p = 0.03$). There were no significant correlations between leptin levels and age, LVEF, serum creatinine, fasting blood glucose, cholesterol, LDL, HDL, triglycerides, and BNP levels. The VE/VCO_2 slope correlated with leptin/BMI ($r = 0.4$; $p = 0.008$), CRP ($r = 0.4$; $p = 0.01$), and leukocyte count ($r = 0.34$; $p = 0.03$). There were no correlations with BMI and waist circumference.

In our study, 19 patients had VE/VCO_2 slope ≥ 35. Leptin levels and BMI-adjusted leptin levels (leptin/BMI) were significantly higher in those patients compared to the rest of the CHF group (leptin –12.9 ± 9.6 *vs* 7.4 ± 5.1 ng/mL; $p = = 0.04$ and leptin/BMI –0.47 ± 0.4 *vs* 0.25 ± 0.1; $p = 0.03$). Peak VO_2 correlated inversely with leptin/BMI ($r = –0.38$; $p = 0.001$), and with the percentage of neutrophils ($r = –0.38$; $p = 0.04$), and positively with the percentage of lymphocytes ($r = 0.32$; $p = 0.045$). There were no correlations with BMI and waist circumference. Twelve patients had peak $VO_2 < 14$ mL/kg/min. Leptin levels and BMI-adjusted leptin levels were significantly higher in these patients compared to the rest of the group (leptin –14.2 ± 10.4 *vs* 8.0 ± 5.8 ng/mL; $p = 0.04$ and leptin/BMI –0.49 ± 0.4 *vs* 0.28 ± 0.2; $p = 0.04$).

In a multivariate step forward regression analysis ($R^2 = = 0.30$; $p < 0.004$) with inclusion of peak VO_2, BMI, waist circumference, leukocyte count, and hsCRP, only peak VO_2 was independently associated with leptin levels ($\beta = –0.29$; $p = 0.04$). There was a trend towards leukocyte count ($\beta = = 0.29$; $p = 0.058$). After adding VE/VCO_2 slope to the multivariate regression analysis model ($R^2 = 0.41$; $p < 0.004$) only VE/VCO_2 slope was independently associated with leptin levels ($\beta = 0.5$; $p = 0.03$).

DISCUSSION

Our data show that peak VO_2 and VE/VCO_2 slope are independently related to leptin levels. Leptin correlated with hsCRP and leukocyte count which are markers of inflammation.

Leptin is the product of the adipocyte *ob*-gene associated with energy expenditure and weight loss. We found markedly elevated serum levels of leptin in patients with CHF compared to healthy control subjects. Because leptin levels are closely related to body fat tissue mass [9, 10], we compared leptin levels corrected for BMI and found also that these levels were significantly higher in CHF patients. In addition, leptin levels and leptin/BMI were significantly higher in patients with BMI < 25 kg/m^2 than in controls, despite the comparable BMI and waist circumferences. This confirms the previous findings of Schulze et al. [3] and Doehner et al. [4].

In our study, serum concentrations of leptin correlated inversely with peak VO_2, with higher levels of leptin associated with more severe exercise intolerance in CHF patients, and this correlation was independent of BMI. Leptin levels

also correlated significantly with another marker of CHF severity: VE/VCO_2 slope. Both are established prognostic markers in CHF patients, with VE/VCO_2 slope being considered the stronger of the two. Wolk et al. [10] found an independent relationship between VE/VCO_2 slope and plasma leptin levels in non-cachectic CHF patients. Furthermore, independently of leptin levels, body fat was a negative predictor of VE/VCO_2 slope. In a separate multivariate analysis, they found that leptin level was no longer associated with lean peak VO_2. They hypothesised that leptin may be a link between metabolic, cardiovascular and respiratory abnormalities in CHF. It may indicate that leptin may play a role in the progression of CHF symptoms.

In our study, peak VO_2 and VE/VCO_2 slope correlated with the inflammatory markers. Furthermore, we found significant correlations between leptin levels and hsCRP and leukocyte count. Low grade inflammation is considered to play an important role in the development and progression of heart failure, together with some other peripheral abnormalities such as metabolic abnormalities, or skeletal muscle alterations [11]. Patients with severe CHF have low peak VO_2 accompanied by increased levels of proinflammatory cytokines [12, 13]. Schulze et al. [3] observed elevated leptin levels and levels of leptin corrected for BMI in patients with advanced CHF (peak VO_2 < 14 mL/kg/min) when compared to less severe CHF and healthy controls. In this subgroup of patients, they found significant correlations between levels of leptin corrected for BMI and TNF-α levels. In our study, hsCRP — an inflammatory protein produced in the liver in response to interleukin-6 stimulation, leukocyte count and erythrocyte sedimentation rate were measured as parameters of inflammation. Levels of hsCRP and leukocyte count were associated with leptin levels and levels of leptin corrected for BMI in CHF patients. Our findings don't allow us to state that leptin is responsible for higher levels of other inflammatory markers in CHF patients or, inversely, that inflammation is stimulating leptin levels.

Leptin receptors have been found in various tissues, which suggests that besides its central effect on food intake, it may also exert peripheral actions [2]. The expression of leptin can be induced, among others, by cytokines [3]. The exact role of leptin in cardiovascular pathophysiology is still not fully understood. Many clinical studies support the concept that high leptin levels are associated with poorer cardiovascular prognosis [14, 15]. Shamsuzzaman et al. [16] demonstrated that leptin and CRP levels are independently associated in normal humans, providing further evidence linking metabolic and inflammatory cardiovascular disease mechanisms. Our findings suggest that leptin may participate in inflammation leading to increase in severity of CHF, as assessed by decreased peak VO_2.

CONCLUSIONS

Elevated serum leptin levels in patients with chronic heart failure are related to peak VO_2, VE/VCO_2 slope and levels of inflammatory markers.

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Leptyna i zapalenie u pacjentów z przewlekłą niewydolnością serca

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Streszczenie

Wstęp: Rola leptyny w patofizjologii chorób układu sercowo-naczyniowego cieszy się rosnącym zainteresowaniem; sugeruje się m.in. jej znaczenie prozapalne. W wielu badaniach donoszono o podwyższonych stężeniach leptyny u pacjentów z przewlekłą niewydolnością serca (CHF) bez kacheksji. Jej znaczenie w tej grupie chorych pozostaje jednak nieznane.

Cel: Celem pracy była analiza stężenia leptyny u pacjentów z CHF i dysfunkcją skurczową lewej komory w zależności od szczytowego pochłaniania tlenu — peak VO_2 i stężeń hsCRP.

Metody: Badana grupa składała się z 41 pacjentów ze stabilną CHF i frakcją wyrzutową lewej komory < 45% (średni wiek 50,2 ± 9,3 roku) i z 8 zdrowych osób tworzących grupę kontrolną (wiek 43,6 ± 14,7 roku). U 16 (39%) pacjentów stwierdzono chorobę wieńcową. U wszystkich uczestników wykonano pomiary antropometryczne (masa ciała, wzrost i obwód pasa), standardowe badanie echokardiograficzne i maksymalny test spiroergometryczny na bieżni ruchomej. Analizowano liczbę leukocytów, wartość OB i stężenia hsCRP. Stężenia leptyny w surowicy mierzono testem RIA.

Wyniki: Stężenia leptyny, w tym stężenia skorygowane względem wartości wskaźnika masy ciała (BMI), były istotnie statystycznie wyższe w grupie pacjentów z CHF niż w grupie kontrolnej (9,2 ± 7,5 *v.* 2,9 ± 1,25 ng/ml; p = 0,005). W grupie CHF stwierdzono istotnie statystycznie wyższe wartości liczby leukocytów, neutrofilów, odsetek limfocytów i stężenia BNP w porównaniu ze zdrowymi ochotnikami. W grupie z CHF zaobserwowano istotne statystycznie korelacje między stężeniami leptyny a BMI (r = 0,55; p < 0,05), obwodem pasa (r = 0,49; p < 0,05), liczbą leukocytów (r = 0,41; p < 0,05), stężeniami hsCRP (r = 0,34; p < 0,05) i peak VO_2 (r = –0,34; p < 0,05). Wieloczynnikowa analiza regresji wykazała, że peak VO_2 było czynnikiem istotnie prognozującym stężenia leptyny. Po uwzględnieniu w wieloczynnikowej analizie regresji parametru VE/VO_2slope, tylko VE/VO_2slope był niezależnie związany ze stężeniami leptyny.

Wnioski: Podwyższone stężenie leptyny u pacjentów z CHF wykazuje związek z peak VO_2, VE/VO_2slope i stężeniami markerów zapalnych.

Słowa kluczowe: przewlekła niewydolność serca, leptyna, białko C-reaktywne, zapalenie, test spiroergimetryczny, VO_2

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