Heart Failure

Leptin and the Ventilatory Response to Exercise in Heart Failure

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OBJECTIVES
The aim of the study was to test the hypothesis that leptin is involved in the regulation of ventilatory responses to exercise in chronic heart failure (CHF).

BACKGROUND
Exercise-induced hyperventilation is a negative prognostic factor in CHF. Studies in animals suggest that leptin, a hormone secreted by adipocytes, contributes to the regulation of respiration. Plasma leptin levels are elevated in non-cachectic CHF, suggesting the possibility that leptin might be involved in dysregulation of ventilation in CHF.

METHODS
We studied 50 patients with stable CHF without cachexia. All subjects underwent anthropometric measurements, resting echocardiography, pulmonary function tests, and a cardiopulmonary exercise test. The ventilatory response to exercise was assessed by calculating the VE/VCO₂ and VE/VO₂ slopes (VE = ventilation per unit time, VCO₂ = carbon dioxide production, VO₂ = oxygen consumption).

RESULTS
Using a multiple regression model, leptin was significantly and positively correlated with both VE/VCO₂ slope (regression coefficient = 0.87, F = 39.32, p < 0.001) and VE/VO₂ slope (regression coefficient = 0.84, F = 24.04, p < 0.001). This correlation was independent of age, gender, body mass index, body fat, ejection fraction, New York Heart Association functional class, pulmonary function, plasma norepinephrine, angiotensin II, brain natriuretic peptide levels, and medications. Also, the greatest VE/VCO₂ slope was seen in subjects in the highest tertile of leptin.

CONCLUSIONS
Leptin is an independent predictor of VE/VCO₂ slope in heart failure, and may be a link between metabolic, cardiovascular, and respiratory abnormalities in CHF.

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Leptin is a 167-amino-acid product of the ob gene, which is produced primarily by adipocytes (1). Although originally associated with the central regulation of satiety and energy metabolism, there is now increasing evidence that leptin may be an important mediator in cardiovascular pathophysiology (2–8). In addition, recent studies in animals suggest that leptin may play an important role in the regulation of respiration, particularly the ventilatory responses to CO₂, and may act both as a neurohumoral modulator of respiratory control mechanisms as well as by producing changes in respiratory mechanics (9–13).

A growing body of literature has described marked ventilatory abnormalities in response to exercise in chronic heart failure (CHF), including a reduced ventilatory efficiency (a high minute ventilation relative to metabolic demand) (14). Ventilatory efficiency during exercise can be assessed by calculating the VE/VCO₂ slope, where VE = ventilation per unit time and VCO₂ = carbon dioxide production. Typically, the VE/VCO₂ slope is increased in patients with CHF in part due to an enhanced ventilatory drive (15,16). Several recent studies have demonstrated that a hyperventilatory response to exercise is a powerful prognostic factor in CHF (15–19). When combined with reduced peak oxygen consumption (VO₂), ventilatory abnormalities identify a subgroup of CHF patients at a particularly high risk of death (16,17,19). Importantly, the VE/VCO₂ slope may be a powerful predictor of event-free survival, while peak VO₂ does not substantially improve the predictive value of the model (16,18). These latter observations speak to the pathophysiologic significance of the mechanisms regulating ventilatory responses to exercise in patients with CHF.

Most clinical studies have reported elevated circulating leptin levels in non-cachectic CHF (20–24). However, the pathophysiologic role of circulating leptin in CHF remains unknown. In the present study we tested the hypothesis that leptin is involved in the regulation of ventilatory responses to exercise in subjects with CHF.

METHODS
Fifty consecutively eligible Caucasian patients with a diagnosis of stable non-cachectic systolic CHF were recruited prospectively at the Mayo Clinic, Rochester, Minnesota. Cardiac cachexia was defined as a body weight <85% of ideal (25). The etiology of CHF was ischemic or nonischemic dilated cardiomyopathy. Patients with conditions likely to influence exercise tolerance independent of CHF (primary lung disease, obesity, musculoskeletal diseases, peripheral vascular disease, chest pain, pacemaker dependency, atrial fibrillation), valvular heart disease, history of...
complex ventricular arrhythmias, or with a moderate smoking history, were excluded. The clinical characteristics of the study group are shown in Table 1. The study was approved by the Mayo Institutional Review Board. Informed consent was obtained before participation.

All subjects underwent anthropometric measurements (including assessment of body fat [26]), resting echocardiography, pulmonary function tests, and a cardiopulmonary exercise test. Left ventricular ejection fraction (EF) was assessed using two-dimensional echocardiography. Pulmonary function measurements were performed at rest and included an assessment of forced vital capacity (FVC) and forced expiratory volume in 1 s (FEV₁). A single-breath diffusion capacity of the lung for carbon monoxide (DLCO) was also measured. Spirometry and DLCO data were collected in accordance with the American Thoracic Society standards (27).

Gas exchange was measured during graded treadmill testing to volitional fatigue. The measurements were obtained using a Medical Graphics metabolic cart validated with classical gas collection techniques. An initial treadmill speed and grade of 2 mph and 0%, respectively, were adjusted every 2 min to yield ~2 metabolic equivalent increase per work level. Anaerobic threshold was determined by the V-slope method (28). Peak VO₂ was defined as the maximal oxygen uptake during exercise. Because of the recent evidence that the adjustment of peak VO₂ to lean body mass increases its prognostic value and allows a more reliable comparison between subjects with variable body mass/fat (because body fat represents metabolically inactive mass) (29), we also measured peak VO₂ corrected for lean body mass (lean peak VO₂). The VE/VCO₂ and VE/VO₂ slopes were calculated below the respiratory compensation point using linear regression analysis. The exercise characteristics are shown in Table 2.

Blood samples were drawn at rest in a supine position. Plasma norepinephrine was measured using high-performance liquid chromatography (ESA Inc., Chelmsford, Massachusetts) (with inter- and intra-assay variability of 3.4% and 3.1%, respectively) and plasma leptin was measured by radioimmunoassay (Linco Research Inc., St. Charles, Missouri) (inter- and intra-assay variability 3.4% to 8.3% and 3.6% to 6.2%, respectively). Brain natriuretic peptide (BNP) was measured by immunoradiometric assay (Shionogi and Co. Ltd., Osaka, Japan) (inter- and intra-assay variability were both 8%) and angiotensin II was measured using a nonequilibrium assay (Phoenix Pharmaceuticals, Belmont, California) (inter- and intra-assay variability were both 8% and 9%, respectively).

### Statistical analysis
In order to assess the independent relationship between leptin and ventilation during exercise, all variables were entered into a multiple stepwise regression model. Peak VO₂ and VE/VO₂ slope, and VE/VCO₂ slope were used as dependent variables. Independent variables included age, gender, body mass index (BMI), body fat,
waist-to-hip ratio, New York Heart Association (NYHA) functional class, EF, plasma norepinephrine, angiotensin II and BNP levels, % predicted FVC, % predicted FEV\(_1\), % predicted DLCO, and leptin. After the initial analysis, medications were added to the model. A value of \( p = 0.05 \) was considered significant.

**RESULTS**

In univariate analysis, leptin was related to VE/VCO\(_2\) slope (Table 3), VE/VO\(_2\) slope \((r = 0.49, p < 0.001)\), and lean peak VO\(_2\) (Table 4). Leptin was also positively correlated with female gender \((r = 0.37, p = 0.008)\), BMI \((r = 0.51, p < 0.001)\), and body fat \((r = 0.73, p < 0.001)\), and negatively correlated with % predicted FVC \((r = -0.29, p = 0.038)\), % predicted FEV\(_1\) \((r = -0.28, p = 0.045)\), and % predicted DLCO \((r = -0.32, p = 0.022)\). Apart from the correlation with leptin, VE/VCO\(_2\) slope was also positively correlated with age, plasma norepinephrine, and BNP, and negatively correlated with the respiratory function tests (Table 3). The VE/VCO\(_2\) slope did not correlate with body fat in univariate analysis. However, the relationship between leptin and VE/VCO\(_2\) slope was further strengthened when leptin levels were adjusted for body fat \((r = 0.50, p < 0.001)\).

When plasma leptin levels were divided into tertiles, the greatest VE/VCO\(_2\) slope was seen in subjects in the highest tertile of leptin (Fig. 1a). In contrast, there was no difference between VE/VCO\(_2\) values in patients divided into tertiles of body fat (Fig. 1b).

In previous publications the threshold value for VE/VCO\(_2\) slope of 34 was used and shown to identify CHF patients at an increased risk of death (15,19). In our study seven subjects had VE/VCO\(_2\) slopes \(\geq 34\). In those subjects leptin levels were higher compared with the rest of the study group, both when expressed in absolute values \((18.0 \pm 10.7 \text{ ng/ml vs. }10.3 \pm 7.6 \text{ ng/ml; } p = 0.022)\) and when expressed as leptin-to-body fat ratio \((0.60 \pm 0.32 \text{ vs. }0.34 \pm 0.20; p = 0.004)\).

In multivariate analysis, leptin was no longer associated with lean peak VO\(_2\) (Table 4), but remained significantly correlated with both VE/VCO\(_2\) slope (Table 3) and VE/VO\(_2\) slope (regression coefficient = 0.84, \(F = 24.04, p < 0.001\)). The positive correlation between leptin and ventilatory responses to exercise was independent of age, gender, NYHA functional class, BMI, body fat (expressed as % body fat or absolute fat mass), EF, plasma angiotensin II and BNP levels, or resting pulmonary function. Consistent with previous reports (30), VE/VCO\(_2\) slope was related to plasma norepinephrine levels, but the association with leptin was independent of norepinephrine. Importantly, VE/VCO\(_2\) slope was significantly and independently correlated not only with absolute levels of leptin, but also with leptin adjusted for body fat (regression coefficient = 0.84, \(F = 38.61, p < 0.001\), body weight (regression coefficient = 0.78, \(F = 29.19, p < 0.001\), and BMI (regression coefficient = 0.77, \(F = 31.58, p < 0.001\)). It should be noted that in multivariate analysis and independent of leptin levels, body fat was a negative predictor of ventilatory responses to exercise.

When medication (angiotensin-converting enzyme inhibitors and angiotensin receptor blockers, beta-blockers, digoxin, and diuretics, each as a separate independent

### Table 3. Association Between VE/VCO\(_2\) Slope and Independent Variables in Univariate and Multivariate Analysis

<table>
<thead>
<tr>
<th>Univariate</th>
<th>Multivariate (Stepwise Regression)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(r)</td>
</tr>
<tr>
<td>Age (yrs)</td>
<td>0.32</td>
</tr>
<tr>
<td>Female gender</td>
<td>0.02</td>
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<tr>
<td>BMI</td>
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<tr>
<td>Body fat (%)</td>
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<tr>
<td>Waist-to-hip ratio</td>
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<td>NYHA class</td>
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<tr>
<td>Leptin (ng/ml)</td>
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</tr>
<tr>
<td>Norepinephrine (pg/ml)</td>
<td>0.31</td>
</tr>
<tr>
<td>Angiotensin II (pg/ml)</td>
<td>-0.20</td>
</tr>
<tr>
<td>BNP (pg/ml)</td>
<td>0.32</td>
</tr>
<tr>
<td>% pred FVC</td>
<td>-0.31</td>
</tr>
<tr>
<td>% pred FEV(_1)</td>
<td>-0.27</td>
</tr>
<tr>
<td>% pred DLCO</td>
<td>-0.29</td>
</tr>
</tbody>
</table>

Blank spaces indicate the variables removed during the stepwise regression process. Beta-coefficient: standardized regression coefficient representing the independent correlation between the respective variable and the dependent variable (after controlling for all other independent variables studied).

Abbreviations as in Table 1.

### Table 4. Association Between Lean Peak VO\(_2\) and Independent Variables in Univariate and Multivariate Analysis

<table>
<thead>
<tr>
<th>Univariate</th>
<th>Multivariate (Stepwise Regression)</th>
</tr>
</thead>
<tbody>
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<td></td>
<td>(r)</td>
</tr>
<tr>
<td>Age (yrs)</td>
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</tr>
<tr>
<td>Female gender</td>
<td>0.05</td>
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<tr>
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<tr>
<td>Body fat (%)</td>
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<tr>
<td>Waist-to-hip ratio</td>
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<td>NYHA class</td>
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<tr>
<td>EF (%)</td>
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</tr>
<tr>
<td>Leptin (ng/ml)</td>
<td>-0.29</td>
</tr>
<tr>
<td>Norepinephrine (pg/ml)</td>
<td>-0.24</td>
</tr>
<tr>
<td>Angiotensin II (pg/ml)</td>
<td>0.12</td>
</tr>
<tr>
<td>BNP (pg/ml)</td>
<td>-0.33</td>
</tr>
<tr>
<td>% pred FVC</td>
<td>0.32</td>
</tr>
<tr>
<td>% pred FEV(_1)</td>
<td>0.27</td>
</tr>
<tr>
<td>% pred DLCO</td>
<td>0.31</td>
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</table>

Blank spaces indicate the variables removed during the stepwise regression process. Beta-coefficient: standardized regression coefficient representing the independent correlation between the respective variable and the dependent variable (after controlling for all other independent variables studied).

Abbreviations as in Table 1.
variable) entered the model, leptin was still independently associated with VE/VCO₂ slope (beta-coefficient = 0.92, F = 47.83, p < 0.001) and VE/VO₂ slope (beta-coefficient = 0.46, F = 18.06, p < 0.001).

We also investigated whether leptin levels were correlated with ventilatory responses during exercise after adding lean peak VO₂ (an index of the severity of heart failure) to the model as an independent variable. Although lean peak VO₂ was significantly (negatively) correlated with ventilatory responses to exercise in multivariate analysis, leptin still remained an independent predictor of VE/VCO₂ slope (beta-coefficient = 0.75, F = 29.64, p < 0.001) and VE/VO₂ slope (beta-coefficient = 0.36, F = 9.78, p = 0.003).

**DISCUSSION**

The novel finding of the present study is the independent relationship between plasma leptin levels and ventilatory responses to exercise in non-cachectic patients with CHF. Specifically, we show that there is a positive correlation between resting plasma leptin levels and VE/VCO₂ slope during exercise, which cannot be explained on the basis of anthropometric measurements, body fat, EF, peak VO₂, other neurohormone levels, pulmonary function tests, or medication. That plasma leptin is not affected by acute exercise (31,32) supports the possibility that leptin levels measured at rest in our study may be directly related to the regulation of ventilation also during exercise.

Leptin circulates not only in its free form, but also in a complexed form, with the soluble leptin receptor being the major leptin-binding protein (33). Therefore, a possible limitation of our study is the fact that we did not measure plasma levels of the soluble leptin receptor. However, the physiologic role of the soluble leptin receptor has not yet been clarified, either in humans or in animal models. On the one hand, it is possible that the circulating leptin receptor binds leptin and acts as an inhibitor of its biologic effects. On the other hand, the soluble leptin receptor may delay leptin clearance in the kidney leading to hyperleptinemia. The bound leptin may then be made available for release and activate leptin responses. In addition, the soluble leptin receptor may play a role in the transport of leptin across the blood-brain barrier, thereby modulating the effects of leptin in the central nervous system. Consistent with this mode of action, an increase in circulating leptin and an increased weight-reducing effect of leptin were both observed in mice overexpressing the soluble leptin receptor (34). Taken together, these observations suggest that, although the soluble leptin receptor may regulate leptin’s availability and bioactivity, its actual role is still unknown and it may potentially either increase or decrease the effects of leptin. Further studies are needed to clarify this issue before the measurements of the soluble leptin receptor can be unambiguously interpreted.

The mechanisms underlying enhanced VE/VCO₂ slope in CHF are complex and probably multifactorial. Several explanations have been proposed, including abnormalities of pulmonary hemodynamics and increased pulmonary dead space (35–38), ergoreceptor overactivity (39), and abnormalities of ventilatory reflex control (40,41). With respect to the latter mechanism, it has been reported that an abnormally elevated VE/VCO₂ slope in CHF correlates with both central and peripheral chemosensitivity (40,41). Interestingly, this relationship was much stronger for central hypercapnic chemosensitivity (40,41). This observation is in agreement with our previous study, which demonstrated a selective potentiation of central chemosensitivity in patients with stable CHF (42).

The effects of leptin on the above mechanisms are essentially unknown. An intriguing possibility is the effect of
leptin on ventilatory control. The influence of leptin on pulmonary mechanics (12) seems unlikely as an explanation for our findings, because in our study the effects of leptin were independent of pulmonary function. It is of note, however, that studies in ob/ob mutant mice have demonstrated that leptin is a powerful respiratory stimulant and leptin deficiency is associated with a depressed hypercapnic ventilatory response (9,13). Leptin replacement in animals with respiratory depression and elevated PaCO2 has been shown to reverse hypoventilation, most likely by stimulation of central respiratory control centers (11). These studies in animals are consistent with the observation that chemoreflex sensitivity to CO2 is selectively potentiated in human obesity (a condition known to be associated with elevated leptin levels) (43). Although significant hypercapnia is not usually seen during exercise, this does not exclude the presence of increased chemosensitivity, whereby a given CO2 level would constitute a stronger ventilatory stimulus. Nevertheless, other mechanisms underlying the association between leptin and ventilation during exercise may also be important and require further studies.

Although age, female gender, norepinephrine, BNP, leptin, and body fat were all associated with VE/VCO2 slope in multivariate analysis, leptin and body fat were by far the most powerful predictors. The strong negative association between body fat (whether expressed as % body fat or fat mass) with ventilation during exercise is of interest. This association was independent of leptin, BMI, or fat distribution, and opposite to the effects of leptin (Table 3), suggesting that body fat (perhaps through some other, as yet unidentified mechanisms) exerts an inhibitory and leptin-independent influence on ventilation during exercise. The benchmark clinical studies, which established the relationship between VE/VCO2 slope and prognosis in heart failure, did not take body fat into account (15–19). Our results showing an inverse correlation between body fat and VE/VCO2 slope, after adjusting for leptin, are consistent with emerging data suggesting an inverse relationship between indices of obesity and clinical outcome in patients with heart failure (44–46).

Leptin has been recently shown to be an independent risk factor for coronary heart disease (47). Elevated VE/VCO2 slope is an important predictor of prognosis in CHF (15–19). The association between leptin and VE/VCO2 slope may thus conceivably contribute, at least in part, to the negative prognostic associations of increased VE/VCO2 slope. Our findings suggest a novel concept in heart failure pathophysiology, namely that leptin, independent of obesity, may be a link between metabolic, cardiovascular, and respiratory abnormalities in CHF.

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