Hyperleptinaemia in chronic heart failure

Relationships with insulin

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Background Leptin, a product of the ob gene, is known to increase energy expenditure. Given that chronic heart failure is a hypercatabolic state, we sought to determine whether congestive heart failure involves elevations in plasma leptin levels. Since leptin secretion is up-regulated by insulin, we also explored whether in congestive heart failure, a hyperinsulinaemic state, plasma leptin levels relate to plasma insulin levels.

Methods Male patients with weight-stable congestive heart failure (n=25, aged 55.5 ± 2.0, mean ± SEM, body mass index=27.4 ± 0.8, radionuclide left ventricular ejection fraction=29.3 ± 3.0%) and 18 controls, matched for age, sex and body fat (dual energy X-ray absorptiometry), underwent measurement of fasting plasma leptin (radioimmunoassay) and insulin levels.

Results Compared to controls, patients with congestive heart failure had higher plasma leptin [8.12 (± 1.12, +1.31) vs 4.48 (± 0.61, +0.70) ng . ml⁻¹, mean ± asymmetrical SEM, P=0.003], 41.5% higher plasma leptin per percent body fat mass (P<0.001), and higher fasting insulin levels [67.8 (± 11.1, +13.3) vs 32.9 (± 5.7, +6.9) pmol . l⁻¹, P=0.010]. In the congestive heart failure group, plasma leptin correlated with total body fat (r=0.66) and fasting insulin (r=0.68) (both P<0.001). In multivariate regression analyses of the congestive heart failure group, fasting insulin (standardized coefficient=0.41, P=0.011) emerged as a predictor of plasma leptin levels, independent of total body fat (standardized coefficient=0.73, P=0.002, R²=0.66, P<0.001).

Conclusions Plasma leptin levels are raised in patients with congestive heart failure. The observation of a positive relationship between plasma leptin and insulin concentrations suggests that the insulin–leptin axis may be related to the increased energy expenditure observed in patients with congestive heart failure.

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Introduction

Leptin, a peptide product of the obese (ob) gene, has been shown to exert thermogenic and anorexigenic effects in mice. Therefore, increasing attention is being focused on the possible involvement of leptin in the regulation of energy balance in humans. Chronic heart failure is a state of increased energy expenditure, in which there is a positive association between plasma leptin concentrations and metabolic rate. It is plausible that in congestive heart failure, a syndrome comprising a panoply of disturbances in anabolic/catabolic metabolism, plasma leptin concentrations may be inappropriately raised, and that plasma leptin concentrations may be related to the hormonal imbalance that characterizes this condition.

Numerous studies have focused on the seemingly direct relationship between body fat plasma leptin concentrations. There is, however, considerable variability in plasma leptin concentrations amongst individuals with comparable degrees of obesity, suggesting that factors other than fat are involved in leptin regulation.
Insulin is known to stimulate ob gene expression\cite{8,9} and although acute hyperinsulinaemia does not affect plasma leptin concentrations\cite{10,11}, prolonged insulin infusions cause elevations\cite{12}. These findings suggest that, in vivo, plasma insulin and, in particular, prolonged hyperinsulinaemia, promotes leptin production.

In this study, we sought to determine whether congestive heart failure involves alterations in plasma leptin concentrations. Having previously shown that congestive heart failure is a hyperinsulinemic state\cite{13,14}, we also explored whether plasma leptin concentrations relate to plasma insulin levels.

**Subjects and methods**

The study group consisted of 25 male patients with congestive heart failure (Table 1). All patients had a body mass index of $\geq 24$ kg m$^{-2}$ and none had noticed or complained of losing weight in the preceding 2 years. The diagnosis of congestive heart failure was based on standard criteria. All patients had been in congestive heart failure for at least 6 months preceding the study. Chronic heart failure was secondary to coronary heart disease in 13 and to idiopathic dilated cardiomyopathy in 12. Concurrent treatments in patients with congestive heart failure included: angiotensin-converting enzyme inhibitors 23, loop diuretics 22, thiazide diuretics 2, potassium-sparing diuretics 7, digoxin 9, and amiodarone 10, either alone or in combination. Healthy controls ($n=18$) were contemporaneous participants of the Heart Disease and Diabetes Risk Indicators in a Screened Cohort Study (HDDRISC)-2, which is a prospective study exploring the relationship of insulin resistance to the subsequent development of coronary heart disease and diabetes mellitus. This study began in 1971 and continues to date. Healthy controls were matched to congestive heart failure patients for age, sex and total body fat (dual energy X-ray absorptiometry). All patients gave written informed consent and the study was approved by the local Ethics Committee.

Patients with chronic lung disease, haemodynamically important valve disease, neuromuscular disorders, myocardial infarction in the preceding 3 months, severe renal failure, symptomatic peripheral vascular disease or excessive alcohol intake were excluded from the study. All congestive heart failure patients underwent a symptom-limited treadmill exercise test for assessment of maximal oxygen consumption (M$\overline{V}$O$_2$) using a respiratory mass spectrometer (Amis 2000, Odense, Denmark) and a standard inert gas dilution technique.

**Laboratory determinations**

Venous blood samples for measurement of fasting plasma leptin were obtained at 0900h, following a 12-h fast. Plasma leptin was measured on duplicate samples using commercially available radioimmunoassay kits (Linco Research, Inc., St Charles, M O, U.S.A.) (intra-assay coefficient of variation=3.43 ± 1.26%). Plasma glucose was determined on the same day using a glucose oxidase procedure\cite{15} and plasma insulin concentrations were measured using a radioimmunoassay\cite{16}.

**Body fat**

Height (m) and weight (kg) were determined to calculate body mass index [weight/height$^2$]. Total and regional fat and lean tissue mass were estimated by dual energy X-ray absorptiometry using a Lunar DPX (Lunar Corp., Madison, WI, U.S.A.)\cite{17}. Total body scans were performed using the fast scan mode (16 cm . min$^{-1}$) unless patient size determined that a slower scan speed (8 cm . min$^{-1}$) should be used, giving radiation doses of 0.05 and 0.1 $\mu$Gy, respectively. Total body scans were analysed using the Extended Research Mode to obtain total and regional fat and lean tissue measurements of the trunk, arms and legs. The trunk region was delineated by an upper horizontal border below the chin, vertical borders lateral to the ribs including all soft tissue but excluding the arms and a lower border formed by oblique lines passing through the hip joints. The leg region was defined as the tissue below the oblique lines passing through the hip joints, and the arm region was defined as the tissue lateral to the vertical borders of the trunk region. All scans were performed and analysed using version 3.6z software. Precision of lean tissue measurements was <2% and fat tissue measurements was <5%\cite{18}.

**Statistical analyses**

All results are presented as mean value ± SEM. Due to skewed distribution, fasting insulin was logarthimically transformed. Given a skewed distribution in the pooled sample, leptin levels were logarthimically transformed for the analyses of group differences. Differences between the groups were analysed using ANOVA. Significant differences were further analysed by Fisher’s exact test. Interrelationships were assessed using simple linear regression (least square method), and multivariate and stepwise regression analyses (SYSTAT, SYSTAT Inc., Evanston, Illinois, U.S.A.). A probability value of <0.05 was considered statistically significant.

**Results**

As shown in Table 1, the groups were well matched for age and both total and regional body fat. Plasma leptin levels were 81.3% higher in patients with congestive heart failure (P <0.003). When leptin concentrations were expressed as the ratio of plasma leptin concentration to the percentage of soft tissue body fat, plasma
leptin levels were 41·5% higher in the congestive heart failure group (P=0·001). Fasting insulin levels were 106·1% higher in patients with congestive heart failure than in controls (P=0·01).

As shown in Table 2, strong correlations emerged between plasma leptin levels and all measures of fat mass, in both the study and control groups. Correlations between plasma leptin and fasting insulin were consistent across the groups (congestive heart failure group r=0·68, P<0·001; controls r=0·73, P<0·01). In pooled subjects, the correlation between plasma insulin and leptin was significant (r=0·81, P<0·0001) (Fig. 1). When outliers (those with log plasma insulin <1·0) were excluded from the pooled group, the correlation between fasting insulin and plasma leptin remained significant (0·68, P<0·001).

In multivariate regression analyses (Table 3), total body fat emerged as the only significant predictor of plasma leptin levels in controls (P=0·001). In contrast, fasting insulin emerged as an additional predictor of plasma leptin levels in patients with congestive heart failure (P=0·011), independent of total body fat. In analysis of pooled subjects, both total body fat (P<0·001) and diagnosis (congestive heart failure or health, P=0·009) emerged as independent predictors of plasma leptin levels. When fasting insulin was entered into this latter model, diagnosis failed to achieve statistical significance (Table 3, pooled subjects, model 2).

### Discussion

We have shown that, compared to controls matched for both total and regional body fat, patients with congestive heart failure are relatively hyperleptinaemic. In accord with our previous observations[13,14], we have found that such patients are also relatively hyperinsulinaemic. The novel finding from this study is that in congestive heart failure, plasma leptin concentrations are strongly related to plasma insulin concentrations, independent of total and regional body fat mass.

In a study of Toth et al.[6], no difference in plasma leptin concentrations was found between patients with congestive heart failure and healthy controls. In this study, however, plasma leptin concentrations in control subjects were higher (6·8 ng . ml$^{-1}$) than those found in our study (4·48 ng . ml$^{-1}$) and in others (3·3 ng . ml$^{-1}$ in non-obese men[19]). Our contrasting findings may also be explained by other factors.
In the study of Toth et al., the study group comprised some cachectic patients, whose plasma leptin concentrations tended to be lower than in non-cachectic patients. The control group was not matched for regional body fat mass. In the present study, the control group consisted entirely of non-cachectic patients of European origin, matched to the study group for both total and regional body fat. Centrality of body fat (waist-to-hip ratio) has been shown to relate positively to plasma leptin concentrations[19] and is therefore a potential confounder. Inclusion of cachectic patients as controls, and the possible confounding effects of central fat upon plasma leptin concentrations might well account for these contrasting findings.

In agreement with our findings, several cross-sectional studies have also found that the positive association between plasma leptin and insulin concentrations is independent of adiposity[19,20] A direct mechanistic link between insulin and leptin is suggested by the finding that insulin stimulates ob gene expression and leptin release[21]. In addition, prolonged insulin infusions have been shown to cause elevations in plasma leptin concentrations[12]. On this basis, the possibility arises that prolonged hyperinsulinaemia may be causally related to elevations in plasma leptin concentrations in patients with congestive heart failure. On the basis of the present study, we cannot discount that insulin resistance, rather than hyperinsulinaemia, is the primary disturbance. Increases in insulin sensitivity produced by the insulin-sensitizing agent troglitazone have been shown to abolish insulin-stimulated leptin release from adipocytes in culture[21]. It is possible therefore that, in

Figure 1  Scatterplot of log10-fasting insulin against log10-plasma leptin concentrations in pooled subjects (○ = CHF, ● = controls).

Table 3 Results of multivariate analyses with plasma leptin levels as the dependent variable

<table>
<thead>
<tr>
<th>Independent variables</th>
<th>Standardized coefficient</th>
<th>P value</th>
</tr>
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<tbody>
<tr>
<td>CHF group</td>
<td>% body fat</td>
<td>0.73</td>
</tr>
<tr>
<td></td>
<td>Fasting insulin</td>
<td>0.41</td>
</tr>
<tr>
<td></td>
<td>R²=0.66, P&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>Controls</td>
<td>% body fat</td>
<td>0.75</td>
</tr>
<tr>
<td></td>
<td>Fasting insulin</td>
<td>0.17</td>
</tr>
<tr>
<td></td>
<td>R²=0.79, P&lt;0.001</td>
<td></td>
</tr>
</tbody>
</table>

F-ratio

| Pooled subjects | 1. % body fat | 65.0 | <0.001 |
|                 | Diagnosis*   | 7.5  | 0.009  |
|                 | R²=0.68      |      |        |
| 2. % body fat   | Diagnosis    | 27.3 | <0.001 |
|                 | Fasting insulin | 2.25 |      |
|                 | R²=0.81      |      |        |

*Diagnosis was entered as a categorical variable (chronic heart failure or health). A general linear model was used for analysis of the pooled sample.
Congestive heart failure, insulin and insulin sensitivity have opposing effects on leptin production. By inference, both hyperinsulinemia and insulin resistance could have similar and perhaps additive effects on leptin production.

In conclusion, we have shown that after correcting for total and regional body fat, patients with congestive heart failure are relatively hyperleptinaemic. The observed relationship between insulin and leptin suggest that insulin, or underlying insulin resistance, may serve as a modulator of plasma leptin in congestive heart failure. Further studies are needed to determine the role of the insulin–leptin axis within the context of the neurohormonal and metabolic disturbances that characterize the syndrome of congestive heart failure.

We are grateful to all the individuals who took part in this study.

References


