Inappropriately low plasma leptin concentration in the cachexia associated with chronic heart failure

D R Murdoch, E Rooney, H J Dargie, D Shapiro, J J Morton, J J V McMurray

Abstract

Background—Cardiac cachexia is a syndrome of generalised wasting which carries a poor prognosis and is associated with raised plasma concentrations of tumour necrosis factor α (TNFα). TNFα increases secretion of leptin, a hormone which decreases food intake and increases energy expenditure.

Objective—To determine whether an inappropriate increase in plasma leptin concentration contributes to the cachexia of chronic heart failure.

Design—Retrospective case–control study.

Setting—Tertiary referral cardiology unit.

Patients—110 human subjects comprising 29 cachectic chronic heart failure patients, 22 non-cachectic chronic heart failure patients, 33 patients with ischaemic heart disease but normal ventricular function, and 26 healthy controls.

Interventions—Measurement of: body fat content by skinfold thickness (cachectic males < 27%, females < 29%); plasma leptin, TNFα, and noradrenaline (norepinephrine); central haemodynamics in chronic heart failure patients at right heart catheterisation.

Main outcome measures—Plasma leptin concentration corrected for body fat content, plasma TNFα and noradrenaline concentration, and central haemodynamics.

Results—Mean (SEM) plasma leptin concentrations were: 6.2 (0.6) ng/ml (cachectic heart failure), 16.9 (3.6) ng/ml (non-cachectic heart failure), 16.8 (3.0) ng/ml (ischaemic heart disease), and 18.3 (3.5) ng/ml (control) (p < 0.001 for cachectic heart failure vs all other groups). Plasma leptin concentration remained significantly lower in the cachectic heart failure group even after correcting for body fat content and in spite of significantly increased TNFα concentrations. Thus plasma leptin was inappropriately low in cachectic chronic heart failure in the face of a recognised stimulus to its secretion. There was no significant correlation between plasma leptin, New York Heart Association class, ejection fraction, or any haemodynamic indices.

Conclusions—Leptin does not contribute to the cachexia of chronic heart failure. One or more leptin suppressing mechanisms may operate in this syndrome—for example, the sympathetic nervous system.

Keywords: heart failure; cytokines; leptin; cachexia

Leptin, the recently identified product of the obesity (ob) gene, is a 167 amino acid hormone that is produced by adipose tissue.1 2 Leptin acts within the brain to decrease food intake, especially fat intake, and increase thermogenesis, probably by inhibiting the synthesis and release of hypothalamic neuropeptide Y.

Dysfunction of this negative feedback loop has been suggested as a mechanism underlying disorders involving abnormalities in body fat content such as obesity, where relative under-production of leptin has been postulated.2 4–6 It is also possible that alterations in the synthesis or release of leptin, leading to its overproduction, may underlie the cachexia that often accompanies chronic medical conditions—for example chronic heart failure, which is characterised by selective loss of adipose tissue. We and others have reported that cachexia in chronic heart failure is associated with increased plasma concentrations of tumour necrosis factor α (TNFα) and other cytokines.7–9 TNFα and interleukin-1 have been reported to increase plasma leptin concentrations in a dose dependent fashion in several species including humans.10–12 Increased plasma leptin concentrations might therefore contribute to cachexia in patients with chronic heart failure, as has been suggested in other wasting conditions such as cancer.13 The present study was designed to test this hypothesis.

Methods

This investigation conforms to the principles outlined in the declaration of Helsinki and was approved by the local committee on medical ethics. All patients gave written informed consent.

PATIENTS

Chronic heart failure

We studied 51 patients (nine female, 42 male), aged 52 to 83 years (mean 66.1), with stable chronic heart failure of more than three months’ duration. All patients had a left ventricular ejection fraction of < 35%, measured by radionuclide ventriculography. None had significant concomitant disease, especially infection, renal or hepatic dysfunction (defined as creatinine > 250 µmol/l or transaminase values more than three times the upper limit of normal, respectively), malignancy, chronic lung disease, thyroid disease, or connective tissue disease. The primary cause of heart failure was coronary artery disease in 48 patients and regurgitant valve disease in three. Twenty five patients were in New York Heart Association (NYHA) class II, 23 in class III, and three in class IV.
Plasma leptin in chronic heart failure

**Ischaemic heart disease**
To exclude an independent effect of ischaemic heart disease on plasma leptin concentration, we studied 33 matched patients (eight female, 25 male), aged 39 to 76 years (mean 59.2), with ischaemic heart disease but normal left ventricular function. All these patients had angiographic evidence of obstructive coronary disease but a normal contrast ventriculogram, and they had reversible myocardial ischaemia on exercise ECG testing or thallium myocardial perfusion imaging.

**Normal volunteers**
We also studied 26 matched healthy volunteers (16 female, 10 male), aged 30 to 76 years (mean 60.1). None had a history of chronic illness or was taking regular drug treatment. All had a normal transthoracic echocardiogram, maximum symptom limited exercise ECG test, and pulmonary function tests.

**BODY FAT ESTIMATION**
A single practised observer measured biceps, triceps, infrascapular, and suprailiac skinfold thickness, in triplicate, by the method of Durnin and Womersley, as previously described. The percentage body fat content was derived from the sum of the mean of these measurements according to standard tables. Patients were defined as cachectic (males with body fat < 27%, females < 29%) or non-cachectic on the basis of percentage body fat content. This definition is based on previously published data on our local population, and has been used in a previous study from our group.

Body mass index (BMI) was also calculated in the usual way (weight (kg) divided by square of the height (m²)).

**SAMPLE COLLECTION AND PEPTIDE ASSAY**

**Blood collection**
Ten millilitres of blood were drawn from an antecubital vein, following an overnight fast and after at least 30 minutes' supine rest, into chilled tubes containing potassium EDTA (1 mg/ml blood) and aprotinin (50 KIU/ml blood). Samples were immediately centrifuged at 4°C and the separated plasma stored at −20°C until assay. All samples were assayed in a single batch within six months of collection in a blinded fashion. Plasma leptin, TNFα, and noradrenaline (norepinephrine) were measured in the same plasma samples.

**Plasma leptin measurement**
Plasma leptin (ng/ml) was assayed, without previous extraction of plasma, using a commercially available direct, specific, monoclonal antibody radioimmunoassay kit supplied by Linco Research Inc (St Charles, Minnesota, USA). The within assay and between assay coefficients of variation are 3.4% to 8.3% and 3.0% to 6.2%, respectively. All assays were run in duplicate and the average of the two measurements reported.

**Plasma TNFα measurement**
Plasma TNFα (pg/ml) was assayed using a commercially available solid phase enzyme amplified sensitivity immunoassay (EASIA) kit supplied by BioSource Europe SA (Fleurus, Brussels, Belgium). The within assay and between assay coefficients of variation are 3.7% to 5.2% and 8.0% to 9.9%, respectively. The minimum detectable concentration and approximate normal range for this assay in healthy subjects are 3 pg/ml and 0 to 20 pg/ml, respectively. All assays were run in duplicate and the average of the two measurements reported.

**Plasma noradrenaline measurement**
Plasma noradrenaline (nmol/l) was extracted from plasma and assayed by high performance liquid chromatography and electrochemical detection as previously described. The within assay and between assay coefficients of variation are both < 10% and the approximate normal range for this assay in healthy volunteers is < 5.0 nmol/l.

**HAEMODYNAMIC MEASUREMENTS**
Forty-two of the patients with heart failure were admitted to hospital as day cases for assessment of central haemodynamic variables by right heart catheterisation. A balloon tipped pulmonary thermodilution catheter was inserted through the femoral vein and advanced to an appropriate position within the pulmonary artery. Following a period of stabilisation of at least one hour, measurements of systemic arterial pressure, right atrial pressure, mean pulmonary artery pressure, and pulmonary capillary wedge pressure were made. Cardiac output was also measured by the thermodilution method and systemic vascular resistance was derived in the usual fashion. An average of at least three of each measurements were taken (five for cardiac output) and the mean recorded.

**STATISTICAL ANALYSIS**
All values are expressed as mean (SEM). Two sample t tests, linear regression analysis, and analysis of covariance were used as appropriate. The two analyses of primary interest (comparison of serum leptin and TNFα between cachectic and non-cachectic chronic heart failure patients) were taken at face value; otherwise the Bonferroni correction was applied to all other analyses to control for multiple comparisons.

To allow for comparison between groups, plasma leptin concentration was corrected according to per cent body fat—that is, corrected plasma leptin = plasma leptin concentration (ng/ml) / %body fat. A probability (p) value of < 0.05 was considered significant.

**Results**
**PERCENTAGE BODY FAT AND BODY MASS INDEX**
Of 51 patients with chronic heart failure, 29 were defined as cachectic according to skinfold measurement derived estimates of percentage body fat. The mean (SEM) body fat content of these patients was 22.2 (1.0)%, compared with 31.0 (0.65)% in the 22 non-cachectic patients. By comparison, BMI was 22.6 (0.61) kg/m² and 29.0 (0.59) kg/m² in these two groups,
Table 1 Results of measured variables in the four groups

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>Normal</th>
<th>IHD</th>
<th>Non-cachectic CHF</th>
<th>Cachectic CHF</th>
</tr>
</thead>
<tbody>
<tr>
<td>60.1 (2.0)</td>
<td>59.2 (1.7)</td>
<td>63.4 (1.4)</td>
<td>68.2 (1.5)</td>
<td></td>
</tr>
<tr>
<td>31.5 (1.4)</td>
<td>28.0 (0.9)</td>
<td>31.0 (0.7)</td>
<td>22.2 (1.0)</td>
<td></td>
</tr>
<tr>
<td>28.6 (1.0)</td>
<td>27.2 (0.6)</td>
<td>29.0 (0.6)</td>
<td>22.6 (0.6)</td>
<td></td>
</tr>
<tr>
<td>18.3 (3.5)</td>
<td>16.8 (3.0)</td>
<td>16.9 (3.6)</td>
<td>6.2 (0.6)**</td>
<td></td>
</tr>
<tr>
<td>Noradrenaline (nmol/l)</td>
<td>–</td>
<td>3.5 (0.3)</td>
<td>4.5 (0.4)</td>
<td></td>
</tr>
</tbody>
</table>

Values are mean (SEM). *p < 0.001 (v normal); †p < 0.001 (v non-cachectic); ‡p < 0.001 (v IHD); †p < 0.006 (v non-cachectic); ‡p < 0.05 (v IHD), corrected for multiple comparisons.

CHF, chronic heart failure; IHD, ischaemic heart disease; TNF, tumour necrosis factor.

respectively. Mean values for percentage body fat in patients with ischaemic heart disease and in healthy volunteers were 28.0 (0.9)% and 31.5 (1.4)% respectively; mean BMI values were 27.2 (0.6) kg/m² and 28.6 (0.95) kg/m² respectively (table 1).

PLASMA LEPTIN CONCENTRATIONS

The mean plasma leptin concentration was 6.2 (0.6) ng/ml in cachectic chronic heart failure patients, 16.9 (3.6) ng/ml in non-cachectic chronic heart failure patients, 16.8 (3.0) ng/ml in ischaemic heart disease patients, and 18.3 (3.5) ng/ml in healthy volunteers. Plasma leptin was significantly lower in the cachectic heart heart failure patients than in non-cachectic heart heart failure patients, ischaemic heart disease patients, or healthy volunteers (p < 0.001 for all). There was no significant difference in plasma leptin concentration between the other groups (table 1).

After correction for body fat content, plasma leptin concentration remained significantly lower in cachectic heart failure patients (0.296 (0.035) ng/ml/% body fat) than in non-cachectic heart failure patients (0.521 (0.095); p = 0.03), ischaemic heart disease patients (0.563 (0.087); p < 0.001), or healthy volunteers (0.529 (0.086); p = 0.02) (fig 1). Plasma leptin concentration was thus found to be inappropriately low in cachectic chronic heart failure—that is, lower than could be accounted for by differences in body fat content.

PLASMA TNFα CONCENTRATIONS

The mean plasma TNFα concentration was 30.5 (2.7) pg/ml in cachectic heart failure patients, 21.6 (1.8) pg/ml in non-cachectic heart failure patients, and 22.7 (1.7) pg/ml in ischaemic heart disease patients.

Figure 1 Corrected plasma leptin concentration (ng/ml / % body fat) according to patient group. Error bars indicate SEM. CHF, chronic heart failure; IHD, ischaemic heart disease.
Plasma leptin in chronic heart failure

Discussion

We have shown that patients with cachectic chronic heart failure have significantly lower plasma leptin concentrations than patients with non-cachectic chronic heart failure or with ischaemic heart disease but normal left ventricular function. A rise in plasma leptin concentration relative to body fat content does not therefore seem to be an aetiopathogenic factor. The cachexia associated with chronic heart failure, assuming normal central transport of leptin and normal leptin receptor sensitivity. In other words, the leptin negative feedback loop appears to be functioning effectively, or even in an exaggerated fashion, in cachectic chronic heart failure patients. Indeed, plasma leptin concentrations in cachectic patients with chronic heart failure are inappropriately low (Fig. 1).

Our findings are to some extent surprising and give new insights into the physiology of leptin control. For example, TNFα concentrations—previously linked to cachexia in various conditions—are increased in chronic heart failure.7–9 TNFα has been shown to increase circulating leptin concentrations in several species, including humans.10–12 Furthermore, other mediators known to increase leptin, such as insulin, growth hormone, and cortisol, are themselves increased in chronic heart failure.14–20 Also, as leptin is cleared from the circulation by the kidneys,21 22 and chronic heart failure is associated with renal dysfunction, plasma concentrations might be expected to increase, as they are in renal failure.23 Despite this, plasma leptin concentrations appear not just low but inappropriately low in cachectic chronic heart failure.

Why might leptin concentrations be reduced in cardiac cachexia in the face of all these influences to the contrary? It has been suggested that cachexia is associated with more deranged cardiac haemodynamics, but this did not explain the difference in leptin concentrations in our cachectic patients. Decreased food intake and increased energy expenditure are known to decrease leptin secretion. The former has never been clearly or consistently implicated in cardiac cachexia.24–27 Recently, however, enhanced energy expenditure has been described in chronic heart failure,28–29 and could play a partial role in cachexia and, perhaps, in leptin suppression. Another possible, and potentially related, explanation is the sympathetic nervous system overactivity that characterises chronic heart failure.30 Adrenergic receptor activation by noradrenaline, for example, decreases leptin release experimentally both in vivo and in vitro.28–30 Acute adrenergic stimulation has also been reported to decrease plasma leptin concentration.31 Recently, isoprenaline infusion has been shown to reduce plasma leptin concentration.32 Our cachectic patients tended to have higher plasma noradrenaline concentrations in keeping with this view, and recently another group has shown that patients with cardiac cachexia have much higher noradrenaline concentrations than non-cachectic patients with chronic heart failure.33 More sophisticated and accurate methods of measuring sympathetic nervous system activity would, however, be needed to test this hypothesis properly.

Interestingly, the administration of leptin deficient mice increases their activity as well as decreasing their food intake.34 It is possible, therefore, that the low plasma leptin concentrations in cachectic chronic heart failure may contribute to the symptoms of fatigue and lethargy which are common in this syndrome. This could also explain the more severe functional limitation observed in cachectic chronic heart failure patients and the higher NYHA class observed in our study.

CONCLUSIONS

In summary, this study shows that the cachexia associated with heart failure is not caused by enhanced leptin release, despite the activation of multiple leptin stimulating pathways in this syndrome. In other words, powerful leptin suppressing mechanisms appear to be operating in chronic heart failure and opposing the effect of factors such as TNFα, insulin, corticosteroids, and growth hormone. One possible explanation for both the low leptin concentrations and increased metabolic rate in cachectic chronic heart failure is activation of the sympathetic nervous system.

The Medical Research Council supports DRM, ER, HJD, JJM, and JYVMcM. This study was presented in part at the 70th Scientific Sessions of the American Heart Association, Orlando, Florida, November 1997, and at the XXth Congress of the European Society of Cardiology, Vienna, August 1998.


Inappropriately low plasma leptin concentration in the cachexia associated with chronic heart failure

D R Murdoch, E Rooney, H J Dargie, D Shapiro, J J Morton and J J V McMurray

Heart 1999 82: 352-356
doi: 10.1136/hrt.82.3.352

Updated information and services can be found at:
http://heart.bmj.com/content/82/3/352

These include:

References
This article cites 30 articles, 9 of which you can access for free at:
http://heart.bmj.com/content/82/3/352#BIBL

Email alerting service
Receive free email alerts when new articles cite this article. Sign up in the box at the top right corner of the online article.

Topic Collections
Articles on similar topics can be found in the following collections

- Drugs: cardiovascular system (8842)
- Epidemiology (3752)

Notes

To request permissions go to:
http://group.bmj.com/group/rights-licensing/permissions

To order reprints go to:
http://journals.bmj.com/cgi/reprintform

To subscribe to BMJ go to:
http://group.bmj.com/subscribe/