Serum leptin concentrations and their relation to metabolic abnormalities in human sepsis

G. L. CARLSON, M. SAEED, R. A. LITTLE, AND M. H. IRVING

North Western Injury Research Centre and Department of Surgery, University of Manchester, Hope Hospital, Salford M6 8HD, United Kingdom

Carlson, G. L., M. Saeed, R. A. Little, and M. H. Irving. Serum leptin concentrations and their relation to metabolic abnormalities in human sepsis. Am. J. Physiol. 276 (Endocrinol. Metab. 39): E658–E662, 1999.—Circulating leptin concentrations are raised in animal models of inflammation and sepsis. The purpose of this study was to determine the effect of sepsis on serum leptin concentration in humans and to examine the relationship between leptin and the metabolic consequences of sepsis. Resting energy expenditure, insulin sensitivity, and fasting serum leptin, plasma insulin, and cortisol concentrations were measured in 20 subjects with intra-abdominal sepsis and 20 healthy control subjects, before and during a 2-h period of euglycemic hyperinsulinemia. Fasting serum leptin concentrations were similar in septic and control subjects. In simple regression analysis, serum leptin concentrations correlated significantly with percent body fat in both septic patients (r = 0.64, P < 0.005) and healthy subjects (r = 0.75, P < 0.0001). Multiple regression analyses additionally indicated that percent body fat, fasting plasma insulin, and plasma cortisol, but not sepsis, were significant and independent determinants of serum leptin concentration. No relationship between leptin and resting energy expenditure or insulin sensitivity was identifiable. A major metabolic role for leptin in human sepsis therefore appears unlikely.

indirect calorimetry; metabolic rate; insulin; cortisol; euglycemic hyperinsulinemia

SEPSIS IS CHARACTERIZED by an increase in resting energy expenditure (REE), impaired utilization of fuel substrate, and resistance to the anabolic effects of insulin (4). The combination of these metabolic abnormalities and the anorexia that accompanies them frequently results in nutritional impairment, even with aggressive nutritional support (34).

The role of counterregulatory hormones and proinflammatory cytokines in the metabolic response to sepsis is well recognized (1, 6). Recent studies, however, have indicated that production and/or plasma concentrations of leptin, the protein product of the ob gene, may be substantially increased by inflammatory or infective stimuli. Administration of endotoxin (14), injection of turpentine (12), or experimentally induced peritonitis in rodents (22) leads to increased mRNA for leptin in adipose tissue and to increased plasma leptin concentration, via interleukin (IL)-1 (12) and tumor necrosis factor (TNF)-α (22)-dependent mechanisms. A recent study of acutely septic patients revealed marked elevation and a loss of the normal circadian rhythm of plasma leptin concentration (2).

Leptin has been implicated in the anorexia associated with animal models of inflammation (14, 30), and administration of leptin to leptin-deficient mice leads to an increase in metabolic rate (26). In addition, although ob/ob mice, which lack the leptin gene, are insulin resistant, hyperleptinemia in humans has been shown to correlate with insulin resistance (31). In vitro studies of isolated rat adipocytes (23) or soleus muscle (19) and human tumor cell lines (7) have demonstrated a direct inhibitory effect of leptin on insulin-stimulated metabolic processes and insulin signaling. Although while there is presently little evidence to suggest that leptin plays a role in the regulation of energy balance or insulin sensitivity in humans, the relationship between hyperleptinemia and the metabolic consequences of sepsis has not been assessed. Furthermore, although acute increases in plasma insulin concentration in healthy humans (17, 28), unlike rodents (29), do not appear to lead to increased leptin concentrations, it is unclear whether this still the case in sepsis. The aims of this study were therefore to confirm the effect of sepsis on leptin concentration in humans, to determine whether changes in REE and insulin sensitivity in sepsis might reflect changes in serum leptin concentration, and to determine whether sepsis modified the effect of hyperleptinemia on serum leptin.

MATERIALS AND METHODS

Patients and control subjects. Twenty adult patients with intra-abdominal sepsis were studied within 96 h of transfer to the Intestinal Failure Unit of Hope Hospital, Salford, UK. All patients had intra-abdominal sepsis proven by clinical examination or radiological studies and had been receiving inpatient hospital treatment for between 14 and 38 days. The cause of the sepsis in 11 cases (55%) was perforated diverticular disease or colorectal anastomotic dehiscence and in five cases (25%), gastroduodenal or gallbladder perforation. Two patients (10%) had a pyonephrosis, and one patient (5%) had a splenic abscess. One patient (5%) had an intra-abdominal abscess without an identifiable cause. For each patient, the severity of sepsis was measured with the sepsis severity score of Elebute and Stoner (11), which takes into account the nature and site of the infection, bacteriology, body temperature, secondary effects (e.g., jaundice), and various hematological and biochemical variables such as white cell count and plasma albumin concentration. The septic patients were compared with 20 apparently healthy volunteers who acted as control subjects. All control subjects were assessed by a medical practitioner and after physical examination and hematological and biochemical investigation were found to be entirely healthy. They therefore had sepsis scores of zero (Table 1).
Parenteral nutrition. Septic patients received parenteral nutrition via a dedicated central venous feeding line for ≥4 days before study. In each case, the feeding regimen provided ≥30 kcal·kg⁻¹·day⁻¹ and 1 g N·120 kcal⁻¹·day⁻¹ with added electrolytes, vitamins, trace elements, and heparin made up in 0.9% sodium chloride and administered over 18–24 h. One-half of the nonprotein calories were administered in the form of Intralipid (Pharmacia, Milton Keynes, UK). Additional electrolytes were provided on a daily basis according to individual clinical requirements. In all cases, parenteral nutrition was discontinued for between 10 and 11.5 h before study. Patients with diabetes mellitus, thyroid disease, and receiving β-adrenoceptor antagonists or other drugs known to affect metabolic rate were excluded from the study. All subjects abstained from nicotine, caffeine, and all nutrients for 10 h before study. Relevant data from patients and control subjects are tabulated in Table 1.

Study protocol. The study protocol was approved by the local research ethics committee, and informed consent was obtained before each study. All studies were performed between 0800 and 1200 h. Subjects were weighed and measured in bedclothes after voiding. All subjects were weighed with a Deltatrac open circuit indirect calorimeter (Datex, Helsinki, Finland) and with the use of the tables of Durnin and Womersley (10). All measurements were made by one observer (M. Saeed) trained in the techniques. A cannula for sampling was inserted into a superficial vein of the dorsum of the hand, which was then placed in a warming box (air temperature 60°C) to produce arterialization of venous blood by warming the hand to 42°C (24). Skin temperature was monitored with a thermistor taped to the dorsum of the hand (Vickers, Hampshire, UK). A second cannula for infusion of insulin and glucose was then inserted into an antecubital fossa vein, and the hand to 42°C (24). Skin temperature was monitored with a thermistor taped to the dorsum of the hand.

Indirect calorimetry. REE measurements were performed with a Deltatrac open circuit indirect calorimeter (Datex, Helsinki, Finland) and were expressed as an average of continuous measurements made over a 30-min period.

Euglycemic-hyperinsulinemic clamp protocol. Both septic and control subjects were studied during a 2-h period of euglycemic hyperinsulinemia with the euglycemic-hyperinsulinemic clamp technique (9). Two rates of insulin infusion were selected to maximize the possibility of detecting insulin-stimulated increases in serum leptin concentration in sepsis. Briefly, a primed continuous infusion of Humulin S (Eli Lilly, Basingstoke, Hampshire, UK) was administered at either 40 μU·m·min⁻¹ (9 control, 10 septic subjects) or 240 μU·m·min⁻¹ (11 control, 10 septic subjects). Arterialized venous glucose concentration was monitored every 5 min throughout the clamp (Beckman Instruments, Fullerton, CA) and maintained at 4.5 mmol/l by adjusting the rate of infusion of a solution of 20% dextrose (Steriflex BP, 20% dextrose, Boots, Nottingham, UK). Insulin sensitivity was measured as the steady-state glucose infusion rate (GIR) during the last 30 min of the clamp (35), and an insulin sensitivity index (32) was calculated for each subject, defined as steady-state GIR per kilogram fat-free mass (FFM), corrected to plasma insulin concentration during the last 30 min of the clamp.

Measurement of serum leptin, plasma cortisol, and insulin concentrations. Serum leptin, plasma cortisol, and insulin concentrations were measured in arterialized venous blood taken at the beginning of the clamp (time 0) and 60 and 120 min thereafter. Serum samples were allowed to clot on ice, centrifuged, and then stored at −70°C before being batched before assay. Leptin concentration was measured with a RIA kit (Linco Research, St. Charles, MO). All samples were measured in triplicate, and the mean of each series was used in subsequent calculations. Plasma cortisol and insulin concentrations were measured with commercially available RIA kits (IDS cortisol kit, Tyne & Wear; Pharmacia insulin kit).

Statistical analysis. Results are expressed as means ± SD. Differences between means in the two groups were evaluated by Student’s t-test. Correlations between two variables were examined by simple bivariate analysis. Independent associations between serum leptin concentration and REE, age, gender, percent body fat, insulin sensitivity, fasting plasma cortisol and insulin concentrations, sepsis, and sepsis severity were examined by stepwise multiple regression analysis. Dummy variables were used for gender and presence or absence of sepsis. An identical analysis was used to examine the independent effects of age, gender, fat mass, FFM, and sepsis on REE. Repeated-measures analysis of variance (MANOVA) was used to evaluate time effect, insulin (dose) effect, and sepsis-time interaction for the effect of euglycemic hyperinsulinemia on serum leptin concentration. All statistical calculations were performed with an IBM compatible microcomputer running SPSS 7.0 for Windows (SPSS, Chicago, IL). P values <0.05 were taken to be statistically significant.

RESULTS

Data pertaining to the control subjects and septic patients are shown in Table 1. Septic patients had a similar lean body mass but a greater percent body fat (29.9 ± 9.0%) than healthy controls (22.4 ± 5.5%, P < 0.01). Despite attempts to match as adequately as possible the ages of septic patients and the healthy control subjects, the septic patients were also slightly but significantly older (40.5 ± 14.0 yr vs. 50.9 ± 13.2 yr, P < 0.05, Table 1).

REE was almost 16% greater in septic patients (1,865.5 ± 330.2 kcal/day) than in healthy controls (1,570.0 ± 194.8 kcal/day, P < 0.001). When the effects of age, sex, FFM, fat mass, and sepsis on REE were studied by multiple regression analysis, only sepsis was found to have a significant independent effect (β = 0.61, P < 0.001, r² = 0.37).

GIR was significantly greater at the higher than the lower plasma insulin concentration in both septic patients and control subjects, but the GIR at either plasma insulin concentration was significantly less in septic patients than in healthy controls (Table 2). Septic patients thus exhibited a significantly lower insulin sensitivity than healthy controls [8.4 ± 6.6 vs. 4.1 ± 3.5 mg·kg⁻¹·min⁻¹/(μmol/ml) × 100, P =

### Table 1. Details of patients and control subjects

<table>
<thead>
<tr>
<th>Subjects</th>
<th>Age, yr</th>
<th>Sex</th>
<th>Sepsis Score</th>
<th>%Fat</th>
<th>FFM, kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>40.5 ± 14.0</td>
<td>M</td>
<td>0 (0–0)</td>
<td>22.4 ± 9.4</td>
<td>54.3 ± 9.2</td>
</tr>
<tr>
<td>Septic</td>
<td>50.9 ± 13.2*</td>
<td>M</td>
<td>16 (10–27)†</td>
<td>29.9 ± 9.0‡</td>
<td>53.7 ± 9.6</td>
</tr>
</tbody>
</table>

Data are means ± SD except sepsis score, which is given as median (range); n = 20 subjects/groups. FFM, fat-free mass. * P < 0.05, † P < 0.005, ‡ P = 0.002 vs. control.
Table 2. Glucose infusion rates and hormone concentrations in septic patients and healthy controls

<table>
<thead>
<tr>
<th>Subjects</th>
<th>n</th>
<th>Insulin Infusion Rate, mU·m⁻²·min⁻¹</th>
<th>Insulin, μU/ml</th>
<th>Glucose Infusion Rate, mg·kg·FFM⁻¹·min⁻¹</th>
<th>Leptin, ng/ml</th>
<th>Cortisol, mmol/l (t = 0 min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Septic</td>
<td>9</td>
<td>40</td>
<td>t = 0 min</td>
<td>t = 120 min</td>
<td>5.4 ± 6.2</td>
<td>0.5 ± 0.2</td>
</tr>
<tr>
<td>Control</td>
<td>10</td>
<td>40</td>
<td>t = 0 min</td>
<td>t = 120 min</td>
<td>7.5 ± 4.2</td>
<td>0.3 ± 0.2</td>
</tr>
<tr>
<td>Septic</td>
<td>11</td>
<td>240</td>
<td>t = 0 min</td>
<td>t = 120 min</td>
<td>8.4 ± 5.8</td>
<td>0.6 ± 0.2</td>
</tr>
<tr>
<td>Control</td>
<td>10</td>
<td>240</td>
<td>t = 0 min</td>
<td>t = 120 min</td>
<td>6.3 ± 6.4</td>
<td>0.2 ± 0.1</td>
</tr>
<tr>
<td>Control</td>
<td>10</td>
<td>40</td>
<td>t = 0 min</td>
<td>t = 120 min</td>
<td>7.7 ± 5.4</td>
<td>0.6 ± 0.2</td>
</tr>
</tbody>
</table>

Data are means ± SD; n = no. of subjects/group. *P < 0.01, septic vs. control; †P < 0.01, 240 vs. 40 mU·m²·min⁻¹ insulin infusion rate; ‡P < 0.001, septic vs. control.

0.01]. Plasma cortisol concentration in septic patients (0.53 ± 0.18 mmol/l) was significantly greater than in controls (0.22 ± 0.12 mmol/l, P < 0.001.), but no significant differences between the groups with respect to fasting or steady-state clamp plasma insulin were observed. Euglycemic hyperinsulinemia at either insulin infusion rate had no effect on serum leptin concentration in either septic patients or healthy controls (Table 2).

Serum leptin concentration correlated significantly with percent body fat (Fig. 1) in both controls (r = 0.75, P < 0.001) and septic patients (r = 0.64, P < 0.01). The results of stepwise multiple regression analysis with serum leptin as the dependent variable are summarized in Table 3. The only significant predictors of serum leptin concentration were percent body fat, cortisol, and plasma insulin concentration. There was an inverse relationship between cortisol and leptin concentrations. Percent fat explained 35.2% of the variability in serum leptin concentration, whereas plasma cortisol and fasting plasma insulin explained only an additional 9.7 and 7.5%, respectively. Gender, age, REE, sepsis, sepsis severity, and insulin sensitivity had no significant independent influence on serum leptin concentration.

**DISCUSSION**

The expression of the leptin gene in adipose tissue is increased in animal models of inflammation (12, 14, 30), and the increase in circulating leptin concentration observed in these models may contribute to the associated reduction in voluntary food intake. There are clear interrelationships between leptin and inflammatory cytokines. Not only can endotoxin, IL-1, and TNF-α induce leptin in rodents (12, 14, 30), but serum leptin concentrations correlate with soluble TNF-α receptor concentrations in healthy and diabetic humans (20), suggesting that the TNF-α system may regulate leptin. It is unclear what role leptin might play in inflammation and whether the findings in animal models indicate a role for leptin in human inflammatory disease. The results of the present study indicated that sepsis was not associated with a significant change in serum leptin concentration in humans, and the relationship between percent body fat and leptin was not significantly altered. These results are at variance with a recent report indicating a significant increase in circulating leptin in sepsis (2). Bornstein et al. (2) found a threefold elevation of plasma leptin in acutely septic patients but did not report on the duration or severity of sepsis, nor the body composition of their subjects or the feeding regimen. Each of these factors might conceivably have influenced their results. The patients in the present study were studied after transfer to a specialized clinical unit but had been septic for ≥14 days. Although the patients in this study suffered from a moderately severe degree of sepsis as indicated by their sepsis scores, their sepsis could not be said to have been acute. It is possible that the patient populations in these two studies were not comparable. Unfortunately, although soluble TNF-α receptor concentrations have been shown to increase in sepsis and may be of prognostic significance (27), they were not measured in the present study, so the previously reported association between the TNF system and leptin (20) could not be explored. Studies of the effect of surgery on serum leptin concentration have shown a significant rise in response to a variety of surgical stimuli.

![Graph](http://ajpendo.physiology.org/)

**Fig. 1.** Relationship between serum leptin concentration and percent body fat in septic patients (○) and healthy controls (●); n = 20 subjects/group.

Table 3. Multiple regression analysis of factors affecting serum leptin concentration

<table>
<thead>
<tr>
<th>Independent Factors</th>
<th>Model 1</th>
<th>Model 2</th>
<th>Model 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Percent body fat</td>
<td>0.593*</td>
<td>0.665*</td>
<td>0.492*</td>
</tr>
<tr>
<td>Plasma cortisol concentration</td>
<td>-0.321†</td>
<td>-0.314†</td>
<td>-0.322†</td>
</tr>
<tr>
<td>Fasting plasma insulin concentration</td>
<td>0.352*</td>
<td>0.449*</td>
<td>0.524*</td>
</tr>
<tr>
<td>r²</td>
<td>0.322†</td>
<td>0.449*</td>
<td>0.524*</td>
</tr>
</tbody>
</table>

Standard regression coefficients (β) are given with level of significance. r². Multiple coefficient of determination. There was no independent effect of sepsis in addition to effects of percent body fat, cortisol, and insulin. *P < 0.001, †P < 0.05.
leptin associated with anorexia and hyperinsulinemia, but only for the first 24 h postoperatively (33). Prolonged administration of recombinant human IL-1α also causes an increase in serum leptin concentration in humans but only for the first 5 days (15). It is therefore possible that serum leptin in our subjects had been higher at an earlier point in the septic illness but had returned to normal by the time of study, although serum leptin has not been shown to rise in response to acute infective episodes in human immunodeficiency virus patients (13). Alternative explanations for our failure to find increased serum leptin concentrations in sepsis might concern prolonged underfeeding or marked differences in body composition in the control and septic groups. Although underfeeding of our septic patients might have depressed serum leptin concentration, retrospective analysis of the feeding regimen in comparison with the REE measured at the time of study suggests that patients received a mean of 120% of their REE, making this explanation unlikely, although REE could have been higher earlier in the course of their illness.

It is well established that serum leptin concentrations in health are determined by percent body fat (5), and this was confirmed for both septic patients and healthy subjects in the present study. Multiple regression analysis indicated, however, that the presence of sepsis had no independent effect on serum leptin concentration. No significant difference was observed between mean fasting serum leptin concentration in septic patients and healthy controls, despite the fact that septic patients were significantly heavier and had a greater percent body fat than the healthy controls, which one might have expected to be associated with an increase in mean fasting serum leptin concentration. Body composition is, however, notoriously difficult to assess in septic patients (3), and significant fluid retention may have occurred, leading to an overestimation of fat mass in some subjects. Septic patients were also slightly but significantly older than healthy controls. It is unlikely, however, that this could have explained our results. Some studies have shown no univariate relationship between age and leptin concentration (28), whereas others have shown an age-related decline in serum leptin concentration after 60 yr of age (25). In the present study, age had no independent effect on serum leptin concentration, and no statistically significant difference was observed between septic and control groups with respect to the number of subjects over the age of 60 in each group (3 out of 20 in both groups) or the median age of this subgroup (65.0 vs. 64.0 yr, control vs. septic, respectively). The results of the study were also unchanged when the data were analyzed after removal of the results of the older individuals. Similarly, there was a higher ratio of women to men in the septic than in the control group. Although we were unable to demonstrate a significant independent association between gender and serum leptin concentration in the present study, previous reports have indicated that serum leptin concentrations tend to be higher in women than men (16), which adds further weight to the negative findings of the present study.

As expected, REE was significantly increased in septic patients, but the lack of an independent association between leptin and REE does not support a link between leptin and the hypermetabolism of sepsis. Similar findings have been reported in obese and normal human subjects (16, 25).

No acute effect of hyperinsulinemia on serum leptin concentration was observed in this study in either control or septic subjects, even at the higher plasma insulin concentration. Whereas studies in rodents have indicated an acute stimulatory effect of insulin on leptin production (29), our findings confirm the results of human studies that indicate a more chronic regulatory role of insulin on serum leptin concentrations (17, 28) and indicate that even in the presence of sepsis, hyperinsulinemia does not seem to increase serum leptin concentrations acutely. Similarly, the significantly increased plasma cortisol concentrations observed in septic patients in this study were not associated with increased serum leptin concentration, and stepwise regression analysis indicated an inverse association between cortisol and leptin. Although corticosteroids increase leptin gene expression in animals (8) and human adipocytes (36), and increased plasma leptin levels have been described in endogenous and exogenous hypercortisolemia (21), the relationship between cortisol and insulin is clearly complex and subject to other controlling factors. Recent studies in healthy humans have demonstrated an inverse relationship between circulating cortisol and leptin concentrations (18).

No association between leptin concentration and insulin sensitivity was observed in multiple regression analysis. Serum leptin has been shown to correlate positively with the severity of insulin resistance in renal failure, possibly via increased fasting plasma insulin concentrations (32). Our failure to confirm this in the insulin resistance of sepsis may relate to the fact that basal insulin levels were not increased in our septic patients.

In summary, we found no significant changes in serum leptin concentration in a group of parenterally fed patients with established abdominal sepsis, despite increased REE and insulin resistance. This suggests that leptin does not contribute significantly to insulin resistance or hypermetabolism in sepsis.

M. Saeed was supported by a fellowship from the Royal College of Surgeons of England.

Address for reprint requests and other correspondence: G. L. Carlson, North Western Injury Research Centre, Hope Hospital, Salford, M6 8HD, UK (E-mail: gcarlson@fs1.ho.man.ac.uk). Received 4 September 1998; accepted in final form 5 January 1999.

REFERENCES


3. Carlson, North Western Injury Research Centre, Hope Hospital, Salford, M6 8HD, UK (E-mail: gcarlson@fs1.ho.man.ac.uk).