Leptin Alterations in the Course of Sepsis in Humans

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Abstract. Neuroendocrine response to sepsis may be divided into acute and prolonged phase. As leptin is implicated in the stress response, leptin's profile during both phases, and the possible relationships between leptin and the neuroendocrine response to sepsis were investigated. Thirty adult patients with sepsis in an intensive care unit were studied. Blood samples were collected at the acute and the prolonged phases. In acute sepsis, leptin levels were higher in patients than in controls (10.2±2.5 vs. 4.1±1.2 ng/ml, p=0.01) and correlated positively with insulin levels and insulin resistance. A decline in leptin levels was found during prolonged sepsis (from 10.2±2.5 to 6.2±1.7 ng/ml, p=0.001), which was not related to survival (p=0.913). At the onset of sepsis, leptin levels increased in correlation with insulin and insulin resistance, possibly indicating a cause-effect relationship. However, the decline in leptin levels during the prolonged phase of sepsis was not related either to survival or to metabolic and hormonal changes.

Leptin is an adipocyte-derived hormone which, apart from communicating the peripheral metabolic status to the brain and thus regulating food intake and energy expenditure, plays a crucial role in the neuroendocrine stress response (1). Circulating cytokines such as interleukin 6 and tumor necrosis factor α (TNF-α) are implicated in this effect (2). Animal studies have shown that the leptin decline during starvation is associated with hormonal changes, such as suppression of thyroid and reproductive axis and activation of the hypothalamic-pituitary-adrenal axis (3), in an effort to protect the organism during the harmful situation.

Several investigators have studied the implication of leptin to similar host defense mechanisms in humans but to date data are rather conflicting. In anorexia nervosa (4), very low leptin levels were reported, while in diabetic ketoacidosis plasma leptin levels were low at the onset of the event and increased significantly with treatment (5). Early in surgery, low leptin levels were found and the leptin decrease paralleled that of cortisol (6). On the contrary, increased leptin production was noted in end-stage renal failure (7). To explain these discrepancies, it should be noted that circulating leptin levels vary considerably among healthy individuals and correlate positively with body weight both in healthy human volunteers and in obese individuals (8, 9).

Sepsis is a generalized inflammatory condition secondary to infection, which carries high mortality rates among hospitalized patients. Studies by van der Berghe et al., demonstrated that neuroendocrine and metabolic responses to sepsis may be divided in two, clearly separated phases (10). The acute response to the insult is characterized by activated hypothalamic-pituitary-adrenocortical axis, hypersecretion of growth hormone (GH) in the presence of low insulin-like growth factor I (IGF-I), low levels of thyroid hormones and low activity of the gonadal axis. Insulin resistance and subsequent decreased glucose utilization are also typical in this period (11). These alterations are considered beneficial since they reduce energy consumption. With the persistence of the critical condition for more than 8-10 days (prolonged phase), an inhibition of hypothalamic-pituitary function becomes evident and a non-specific wasting syndrome is developed, despite adequate feeding of the patients (10).

Some of these events imitate leptin-dependent effects during starvation in animals as described by Ahima et al. (12) and the intriguing possibility of leptin-induced endocrine response to human sepsis has recently been evaluated. Administration of endotoxin in non-human primates and healthy volunteers resulted in a delayed increase of leptin levels, i.e., 17-24 h post-administration (13), while there was no effect during the first 17 h (13, 14). Patients with acute sepsis were found to have higher leptin levels than controls (15, 16), while the normal reciprocal relationship of the circadian rhythms of leptin and cortisol was abolished. In most cases, patients were studied at the onset of sepsis. Since the acute and prolonged critical
illness present significant differences in pathophysiology (10), the time-dependent alterations of leptin during both the acute and prolonged sepsis were investigated here. The potential relationships between leptin, metabolic and hormonal factors and possible effect on patient survival were also examined.

Patients and Methods

Patients. A total of 30 critically-ill adult patients, (21 men, 9 women), hospitalized in a multidisciplinary intensive care unit (ICU) of a University Hospital were recruited for this study over a period of 6 months. The mean ± SD age was 44.5±15.9 years. The average body weight was 65.8±13.9 with a statistically significant difference between male and female patients (75.8±2.0 vs. 57.5±5.6 kg, p=0.02). All patients fulfilled the criteria of sepsis, as established by the ACCP-SCCM consensus conference, 1992 (17). Eight of the 30 patients entered the ICU because of sepsis, while the remaining 22 patients were admitted to the ICU for other diseases and developed sepsis as a secondary complication. Further inclusion criteria were: age >18 years, no history of psychiatric, endocrine or metabolic disease, no HIV infection, no history of anti-tumor medication or radiation and no treatment with insulin, steroids, thyroid or dopamine before and/or during the study. Once admitted to the ICU, and gastrointestinal tract function permitting, all patients were administered continuous total or partial parenteral and/or enteral nutrition (30-45 Kcal/kg/day) with standard composition (1-1.5g/kg protein, 1g/kg fat and 3-4 g/kg glucose daily) to achieve a protein to non-protein caloric ratio of 1/100. The severity of the patients’ condition was measured according to the simplified acute physiology scoring (SAPS) II system (18). The mean SAPS II score on the ICU admission day was 50.9±3.8.

Study of the acute and prolonged phase of sepsis. Within 24 h after the diagnosis of sepsis was established, blood was withdrawn at 8:00 a.m. in all patients for measurements of leptin, insulin, GH, cortisol, T3, T4, and TSH levels and the usual hematological and biochemical profiles. This was considered to be the baseline evaluation, i.e. during the acute phase of sepsis. For the study of the prolonged phase of sepsis, blood sampling was performed after 14 days and every 5 days thereafter at 8 am until patients were discharged or died.

Patient outcome. Twelve patients (40%), 9 men and 3 women died in the ICU (non-survivors) and eighteen patients (60%), 12 men and 6 women, were discharged to the ward and subsequently left the hospital (survivors). There was no statistically significant difference between non-survivors and survivors in age, weight, and hospitalization time. Nine non-survivors and thirteen survivors developed sepsis while in ICU.

Control subjects. The control group was comprised of 13 healthy adults, age- and gender-matched to the patients. The above-mentioned exclusion criteria were also applied to control subjects. Their mean±SD age was 51.5±11.3 years. The average body weight was 70.1±12.06 kg and no patient was on a caloric-restricted diet or had significant alterations of body weight for at least one month. The blood sampling in controls was performed only once at 8:00 a.m. The study protocol was approved by the Hospital Ethics Committee. Written consent was obtained from next of kin.

Hormone assays. The samples were collected, centrifuged and sera were stored in aliquots at ~20°C until assayed. For determination of leptin, insulin, GH, cortisol, T3, T4, and TSH levels, all samples were processed in duplicate in the same assay run.

Serum leptin concentrations were quantified by a commercially available immunoradiometric assay (IRMA) kit (Diagnostic Systems Laboratories, Inc., Webster, TX, USA). The detection limit was 0.25 ng/mL. The intra-assay co-efficient of variation was 3.7%, 4.9% and 2.6% at leptin concentrations of 2.75, 13.5 and 73.6 ng/mL, respectively. Serum insulin, growth hormone, cortisol, T3, T4 and TSH concentrations were determined with commercially available assays. Blood cell counts and glucose were measured by standard methods in the central hospital laboratory.

The estimate of insulin resistance by the Homeostasis Model (HOMA) score was calculated by the formula: fasting insulin (µU/mL) x fasting glucose (mmol/L) /22.5 (19).

Statistical analysis. The data analysis was performed with the SAS statistical program. For statistical analysis, the acute phase of sepsis data were considered those obtained within 24 h after the criteria for the diagnosis of sepsis were met. As prolonged phase data, data from the day that the maximal difference of leptin from the acute sepsis levels was noted were considered. This sample was taken 21.8±3.5 days after the onset of sepsis and 3.4±3.0 days before the final outcome (death or discharge from the hospital).

Comparisons of different variables between the acute and the prolonged sepsis were made by paired t-test and Wilcoxon’s signed-rank test, when appropriate. Comparisons between patient subgroups were made by an independent samples t-test and Wilcoxon’s rank sum test, when appropriate. Spearman’s correlation coefficient (r_s) was used to evaluate the relationship between variables. In a univariate analysis, Cox regression models were fitted to examine the effect of each single factor to survival. Statistically significant factors were further used in a multiple stepwise Cox regression analysis. The backward elimination method was used for the selection of significant independent predictors. Data are expressed as mean±SD. Two-tailed p<0.05 was considered statistically significant.

Results

As shown in Figure 1, the leptin levels in patients with acute sepsis were higher than in controls (10.2±2.5 vs. 4.1±1.2 ng/ml, p=0.01). Leptin in non-survivors was almost 2-fold higher than in survivors (14.35±7.44 vs. 7.48±1.9 ng/ml, Figure 1) but this difference did not reach statistical significance. A statistically significant decline in patient leptin levels was found during prolonged sepsis (from 10.2±2.5 to 6.25±1.7 ng/ml, p=0.001). This drop was greater in non-survivors than in survivors (6.2±4.4 and 3.2±1.1 ng/ml reduction from acute sepsis levels, respectively), but without statistically significant difference and as shown in Figure 1 there was no difference in leptin levels between the two groups during prolonged illness.

As indicated in Table I, during acute sepsis, patients showed significantly lower T3 and T4 as compared to controls (57.5±18.3 vs. 88.6±10.1 ng/dl, p=0.01 for T3; 4.9±1.4 vs. 6.2±2.3 µg/dl, p=0.003 for T4), while non-
survivors exhibited significantly lower T3 and T4 levels than survivors (46.6±14.9 vs. 68.6±14.7 ng/dl, p=0.001 for T3; 4.0±0.8 vs. 5.8±1.3 µg/dl, p=0.0008 for T4). At prolonged sepsis, in non-survivors T3 and T4 remained at the same low levels and, furthermore, TSH declined (from 0.9±0.3 to 0.3±0.1 µU/ml, p=0.01; Table I); in survivors, an increase of the aforementioned parameters was noted and reached significance in T3 (from 68.6±14.7 to 85±19.4, p=0.04 during acute and prolonged sepsis, respectively).

There were no significant differences in cortisol levels between patients with acute sepsis and controls, or between survivors and non-survivors, although the latter had higher levels (Table I). Cortisol levels further increased during prolonged sepsis in non-survivors and reached statistical significance when compared to survivors (25.9±7.0 vs. 17.3±6.2 µg/dl, p=0.002, Table I).

Insulin and insulin resistance as expressed by HOMA were significantly elevated in patients compared to controls, during both the acute and prolonged sepsis. No difference was noted in glucose, insulin and insulin resistance in any patients between acute and prolonged sepsis (Table I). During prolonged sepsis however, the non-survivors showed significantly higher glucose levels as compared to the survivors (242.5±62.1 vs. 115.7±80.9 mg/dl, respectively, p=0.027, Table I).

Correlation analysis disclosed a positive correlation between leptin and insulin levels (r_s=0.599, p=0.0007) as well as leptin levels and insulin resistance expressed by HOMA (r_s=0.549, p=0.02), during the acute phase of sepsis. In prolonged sepsis, both aforementioned relationships were abolished. Moreover, no significant correlation between leptin and T3, T4, TSH, glucose, cortisol or GH was demonstrated at either time-point.

To examine the effect of different parameters on survival, the Cox regression model analysis was performed. In univariate analysis, T3 and T4 were strongly and positively related to survival, while the presence of lymphopenia and insulin resistance as expressed by HOMA were negatively related (Table II). However, in multivariate analysis adjusting for all parameters with the backward elimination procedure, only T4 was found to be positively related to survival.
Furthermore, as revealed by Cox proportional hazards model analysis, the decrease in leptin levels at the prolonged phase of sepsis was not related to survival \((p=0.913)\).

**Discussion**

It has been postulated that during sepsis leptin does not act as a messenger of body fat stores in the brain, but as a stress-related peptide regulating the host response to injury \((2)\). As the acute and prolonged critical illness are characterized by clearly different neuroendocrine and metabolic alterations \((10)\), we decided to investigate the time-dependent alterations of leptin during both acute and prolonged sepsis in the same patient group. Thus, the evaluation time was carefully selected so that acute and prolonged phases of sepsis were clearly represented and discriminated in all our patients.

We demonstrated that the acute sepsis (within 24 h from the onset of sepsis) patients exhibited higher leptin levels compared to controls, while during the prolonged phase (>2 weeks from the onset of sepsis) a statistically significant decline of leptin was revealed. Arnalich et al. \((15)\) studied critically ill septic patients for 48 h after the onset of sepsis and demonstrated increased leptin levels in the acute phase that declined in those patients who recovered within the 48-h study period. Borstein et al. \((16)\) also reported higher leptin levels in patients evaluated during acute sepsis only. Furthermore, Van de Berge et al. \((20)\) demonstrated that in protracted critical illness leptin levels were similar to controls. However, in the latter study patients were not evaluated during acute sepsis. Thus, our present study provides novel information on the alterations of leptin levels in both acute and prolonged sepsis in the same cohort of critically ill septic patients.

Papatheonasogolou et al. \((21)\) studied 35 critically ill patients with systemic inflammatory response syndrome (SIRS) \((17)\) and multiple organ dysfunction for a period of 14 days. They reported no differences in baseline leptin levels between patients and matched case controls, while the patients’ leptin levels progressively increased from the first to the last study day \((21)\). The results from our study do not necessarily contradict the aforementioned data. Differences

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### Table I. Biochemical and hormonal variables in patients with sepsis.

<table>
<thead>
<tr>
<th></th>
<th>Controls (n=13)</th>
<th>Septic patients (n=30)</th>
<th>Survivors (n=18)</th>
<th>Non-survivors (n=12)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Acute sepsis</td>
<td>Prolonged sepsis</td>
<td>Acute sepsis</td>
<td>Prolonged sepsis</td>
</tr>
<tr>
<td>WBC</td>
<td>13711±5404</td>
<td>14179.4±6704</td>
<td>13420±6249</td>
<td>11891±3317</td>
</tr>
<tr>
<td>Lymph</td>
<td>1324.8±943</td>
<td>1491.1±1151.1</td>
<td>1608.7±1036</td>
<td>2058±1242</td>
</tr>
<tr>
<td>T3 (ng/dl)</td>
<td>88.6±10.1</td>
<td>75.9±31.9</td>
<td>68.6±14.7</td>
<td>54.6±49.6</td>
</tr>
<tr>
<td>T4 (ng/dl)</td>
<td>6.2±2.3</td>
<td>5.87±2</td>
<td>5.8±1.3</td>
<td>4.6±2.1</td>
</tr>
<tr>
<td>TSH (µIU/ml)</td>
<td>1.4±1.1</td>
<td>1.3±1.1</td>
<td>1.4±0.9</td>
<td>0.9±0.3</td>
</tr>
<tr>
<td>Cortisol (µg/dl)</td>
<td>15.1±4.8</td>
<td>19.4±7.2</td>
<td>22.9±15.1</td>
<td>25.9±7.0</td>
</tr>
<tr>
<td>Glucose (mg/dl)</td>
<td>97±5.8</td>
<td>145.9±75.1</td>
<td>138.6±67.8</td>
<td>242.5±62.4</td>
</tr>
<tr>
<td>Insulin (µIU/ml)</td>
<td>11.6±1.1</td>
<td>43.9±41§</td>
<td>33.4±12.3°</td>
<td>30.1±19.8</td>
</tr>
<tr>
<td>HOMA</td>
<td>2.7±3.1</td>
<td>17.0±7.2*</td>
<td>12.6±6.8°</td>
<td>22.8±20.2*</td>
</tr>
<tr>
<td>GH (ng/ml)</td>
<td>1.8±4.3</td>
<td>3.3±4.1</td>
<td>5.2±5.2</td>
<td>4.9±6.7</td>
</tr>
</tbody>
</table>

\(a\)statistically significant difference from controls; \(b\)statistically significant difference from survivors at the same time-point; and \(c\)statistically significant difference from acute sepsis values within the same subgroup.

WBC, white blood cells; TSH, thyroid secreting hormone; HOMA, Homeostasis Model score; GH, growth hormone.

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### Table II. Simple Cox regression models of survival in septic patients.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Relative Risk</th>
<th>p-value</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>0.99</td>
<td>0.689</td>
<td>0.96-1.03</td>
</tr>
<tr>
<td>Lymphopenia</td>
<td>0.14</td>
<td>0.010</td>
<td>0.03-0.63</td>
</tr>
<tr>
<td>Leptin</td>
<td>0.96</td>
<td>0.181</td>
<td>0.91-1.02</td>
</tr>
<tr>
<td>GH</td>
<td>1.06</td>
<td>0.312</td>
<td>0.95-1.18</td>
</tr>
<tr>
<td>F</td>
<td>0.97</td>
<td>0.252</td>
<td>0.91-1.03</td>
</tr>
<tr>
<td>Glucose</td>
<td>0.99</td>
<td>0.238</td>
<td>0.98-1.01</td>
</tr>
<tr>
<td>Insulin</td>
<td>0.98</td>
<td>0.187</td>
<td>0.96-1.01</td>
</tr>
<tr>
<td>HOMA</td>
<td>0.16</td>
<td>0.021</td>
<td>1.02-1.31</td>
</tr>
<tr>
<td>T3</td>
<td>1.06</td>
<td>0.007</td>
<td>1.02-1.10</td>
</tr>
<tr>
<td>T4</td>
<td>4.78</td>
<td>0.001</td>
<td>1.85-12.35</td>
</tr>
<tr>
<td>TSH</td>
<td>1.52</td>
<td>0.17</td>
<td>0.83-2.81</td>
</tr>
</tbody>
</table>

GH, growth hormone; HOMA, Homeostasis Model score; TSH, thyroid secreting hormone.
in the selection criteria of the patients studied, in their inclusion day and in their follow-up duration may explain the apparent discrepancies of the two reports. Patients of the Papathanassoglou et al. study had SIRS (i.e. systemic inflammation non-related to infection (17)) and not sepsis, while the follow-up period lasted 14 days after baseline (21). In contrast, all our patients were septic (i.e. had systemic inflammation secondary to infection (17)), and their initial estimation was performed at the very early onset of sepsis; in addition, follow up evaluation was extended to 25.2±3.6 days, a period which may allow for a more profound development of the neuroendocrine alterations seen in prolonged sepsis (10).

A possible explanation for the biphasic response of leptin observed in our patients during the course of sepsis could be that increased release of inflammatory cytokines during the acute phase is responsible for the initial augmentation of leptin levels (15, 21, 22). During prolonged sepsis, other mediators such as decreased activity of the GH axis and concomitant low IGF-I levels (20) may be responsible, at least in part, for the observed leptin decline. Glucocorticoids acutely increase leptin secretion in animals and humans (23, 24) and therefore the activation of the hypothalamo-pituitary adrenal axis during the stressful period of sepsis was proposed (16) to increase leptin secretion. However, no correlation was demonstrated between leptin and cortisol levels, either in the acute or in the prolonged septic phase in our study and elsewhere (20), indicating that steroids do not participate in the described leptin alterations.

Insulin resistance, expressed by HOMA, was present during both phases of sepsis as previously shown (11). In our study, at the onset of sepsis leptin correlated positively with insulin levels and insulin resistance, indicating a cause-effect relationship which, surprisingly, was lost during prolonged sepsis.

It was hypothesized that the increase of leptin levels during the acute phase of sepsis may have a protective effect on the host defense (15). In our study, and in agreement with the results of Papathanassoglou et al. (21), we did not find differences in leptin levels between survivors and non-survivors, during either the acute or the prolonged phase of sepsis, although a trend for higher leptin levels in non-survivors was observed. In contrast, a correlation between leptin and survival in septic patients was previously reported (15, 16). As already proposed (21), any effect of leptin on the outcome of septic patients may be confounded by the concomitant changes of other mediators, such as cytokines and hormones, and thus the use of multivariate analysis is necessary. Using this statistical model, we demonstrated that survival was not related either to absolute leptin levels, or to the decline of leptin during prolonged sepsis, as reported previously (21).

In conclusion, in our study population, increased leptin levels, positively related to insulin levels and insulin resistance, characterized the acute phase of sepsis, possibly indicating a cause-effect relationship. However, during prolonged sepsis, a decline of leptin levels and persistence of insulin resistance were noted. During the latter period, the non-survivors exhibited increased serum cortisol, lower thyroid hormones and TSH levels and increased glucose, denoting deterioration of insulin resistance. Leptin levels and their alterations during sepsis did not affect the survival of septic patients.

References


Tzanela et al: Leptin in Sepsis

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