Serum leptin and ghrelin correlate with disease activity in ANCA-associated vasculitis

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Objectives. To study serum levels of leptin and ghrelin in ANCA-associated vasculitis (AAV).

Methods. Thirty-seven patients with AAV (21 patients with active AAV at initial presentation and during follow-up, 16 patients with AAV in long-term remission) and 21 matched healthy controls were included. Serum levels of leptin and ghrelin were measured at 0, 6 and 12 months by radioimmunoassay. Disease activity was gauged by Birmingham Vasculitis Activity Score (BVAS), CRP and circulating endothelial cells (CECs).

Results. Leptin levels were significantly lower in patients than in healthy controls (9.1 vs 29.4 ng/ml; P < 0.005) and declined to normal values at 12 months (306.4 ± 36.2 pmol/l). There was a significant positive correlation between ghrelin levels and disease activity, whereas leptin levels were negatively correlated with disease activity (CRP, BVAS and CECs). Accordingly, correlations between the ghrelin/leptin ratio and markers of disease activity reached the highest level of significance (all P < 0.001).

Conclusions. Active AAV is characterized by decreased serum leptin and increased serum ghrelin, both of which return to normal with successful therapy. The role of leptin and ghrelin during the pathogenesis of AAV and the effects of these peptides on endothelial cells warrant further study.

Key words: ANCA-associated vasculitis, Leptin, Ghrelin, Circulating endothelial cells.

Introduction

Leptin, a 16 kDa peptide hormone of the long chain helical cytokine family, is predominantly produced by white adipocytes [1]. Initially described as a hormone that regulates food intake and energy balance, leptin is now regarded as a pivotal factor in the interplay between neuroendocrine function and the immune system. Circulating leptin exists as free and protein-bound hormone and the role of bound leptin remains uncertain [2]. Effects of leptin vary considerably from one pathophysiological context to another: during acute inflammation, pro-inflammatory cytokines increase circulating leptin, which in turn triggers cytokine release in monocytes/macrophages and stimulates T-cell mediated immunity [3, 4]. Recent models and in vivo observations, however, suggest that leptin may also limit the inflammatory response [5, 6]. Ghrelin, another 28-amino acid peptide recently isolated from human gastric epithelial cells, is the natural ligand for the growth hormone secretagogue receptor (GHS-R) [7]. One of the most important biological activities of ghrelin is the stimulation of food intake during the long-term regulation of body weight [8]. Evidence has emerged to suggest that ghrelin also exerts multiple immunoregulatory effects [9]. In this regard, several in vitro studies demonstrate an anti-inflammatory effect of ghrelin while other studies have shown pro-inflammatory properties of the peptide [10, 11]. It is currently believed that ghrelin and leptin exert mutually reciprocal regulatory effects within the immune system [9, 10]. To our knowledge, the leptin/ghrelin system has not been studied in the ANCA-associated vasculitides (AAVs). These disorders have a clear pro-inflammatory phenotype; hence, involvement of the leptin/ghrelin system through pro-inflammatory pathways is conceivable. Inflammatory phenomena abate with successful therapy during the course of vasculitides. The vasculitides thus provide an opportunity to study the leptin/ghrelin system in relation to various intensities of inflammatory disease. Finally, AAVs are frequently accompanied by anorexia and weight loss, which may be mediated by the leptin/ghrelin-system. We performed a clinical study to evaluate serum levels of free and bound leptin and ghrelin during the course of AAV and correlate serum levels of the two peptides with disease activity.

Methods

Patients

All patients were recruited from the Department of Nephrology at Hannover Medical School between 2002 and 2006. The study was carried out in accordance with the Declaration of Helsinki and approved by the institutional review board. Informed consent was obtained. The diagnosis of AAV was established in accordance with the Chapel Hill classification [12]. Disease activity was assessed in accordance with the Birmingham Vasculitis Activity Score (BVAS) [13]. The diagnostic criteria for WG were typical presentation with involvement of the upper respiratory tract (as described in the ACR criteria for WG) and granulomatous inflammation on histology. The diagnostic criteria for microscopic polyangiitis (MPA) were necrotizing pauci-immune vasculitis or glomerulonephritis of the small vessels without granuloma.

A total of 37 patients were included in the present study. Of those, 21 patients with untreated biopsy-proven active AAV (BVAS ≥6, 16.0 ± 7.4) were assessed at initial presentation and during follow-up at 6 and 12 months of therapy (14 patients had WG and 7 had MPA). Twelve patients had new-onset systemic vasculitis and nine patients had a vasculitic relapse. Exclusion criteria were age under 18 yrs, pregnancy, HIV positivity, malignancies and haemodialysis therapy.

Twenty-one healthy BMI- and age-matched voluntary employees of the Hannover Medical School served as controls. In addition, 16 archival sera of patients with AAV in long-term remission (length of remission ≥24 months, 53.3 ± 49.2 months, BVAS = 0) were analysed at initial presentation and during follow-up at 6 and 12 months of therapy. These patients were off immunosuppression or received maintenance oral prednisolone ≤10 mg/day. Clinical data of patients and characteristics of healthy controls are provided in Table 1.
All patients were treated with 1 mg/kg body weight/day prednisolone orally. By day 15, tapering of the steroid regimen was initiated, with a reduction of 10 mg/week. In all patients with systemic vasculitis, oral prednisolone was started on day 4 after 500 mg prednisolone was given on days 1–3 intravenously. Cyclophosphamide was administered orally or intravenously for 3–6 months followed by azathioprine or mycophenolate mofetil.

**Laboratory testing**

Peripheral blood samples at initial presentation were obtained before or within 24 h of the initiation of treatment. All samples were centrifuged and serum was stored at −80°C. Height and weight were determined and the BMI was calculated. Leucocyte counts, CRP and serum creatinine were measured by standard technique. Glomerular filtration rate (GFR) was estimated using the Cockroft–Gault formula. Circulating endothelial cells (CECs) were enumerated as a laboratory marker of microvascular injury according to a European consensus as described elsewhere [14, 15].

**Radioimmunoassays for the detection of leptin and ghrelin**

Levels of immunoreactive total leptin were measured by commercially available RIA (Human Leptin RIA kit; Linco Research, St Charles, MO, USA) [16]. Serum ghrelin concentrations were determined by RIA as described elsewhere [17]. The ghrelin antibodies exhibited no cross-reactivity with leptin or related peptides, such as motilin and growth hormone–releasing hormone. The detection limit was 34 pmol/l, and the inter- and intra-assay coefficients of variance were 4.1 and 2.6%, respectively. BMI did not change significantly during follow-up.

**Statistical analysis**

Differences between patients and matched healthy controls at the time of the initial presentation were evaluated using Mann–Whitney U-test (two sided). Friedman’s test was used to demonstrate statistical differences in parameters during follow-up and Wilcoxon testing (two sided) was used to show that parameters at baseline, 6 and 12 months were different. Correlations between serum levels and parameters of disease activity were calculated with Spearman’s test. Statistical significance was accepted at 5% probability levels. Data are displayed as mean ± s.d. unless otherwise stated. Data analysis was performed using SPSS software (SPSS Inc., Chicago, IL, USA).

**Results**

At baseline, serum leptin levels were significantly lower in patients with AAV when compared with healthy controls (9.1 ± 6.1 vs 22.3 ± 22.4 ng/ml; P < 0.05). This difference persisted when leptin levels were corrected for BMI [0.35 ± 0.24 vs 0.85 ± 0.83 (ng/ml)/BMI(kg/m²); P < 0.05] (Fig. 1).

Serum leptin, leptinBMI, ghrelin, CECs, BVAS, steroid dose and leucocytes at baseline, 6 and 12 months after initiation of the treatment were different by Friedman’s test (all P < 0.001, except CEC P = 0.025), whereas serum creatinine (P = 0.814) and GFR (P = 0.646) did not vary during follow-up. At initial presentation, 38.1% of patients did not report on weight loss, whereas 28.6 and 33.3% reported on weight loss of ≤2 and ≥2 kg, respectively. However, weight loss before initial presentation did not correlate with leptin, leptinBMI or ghrelin (P = 0.62, P = 0.73, P = 0.81, respectively). BMI did not change significantly during follow-up (P = 0.712).

Mean leptin levels increased significantly after 6 (27.8 ± 21.9 ng/ml; P < 0.001) and 12 months (24.6 ± 21.0 ng/ml; P < 0.001) as compared with baseline values, and were no longer different from healthy controls. BMI-corrected leptinBMI also increased significantly after 6 [1.0 ± 0.8 (ng/ml)/(kg/m²); P < 0.001] and 12 months [0.9 ± 0.79 (ng/ml)/(kg/m²); P < 0.001] as compared with baseline values (Fig. 1). Notably, serum levels of leptin and leptinBMI normalized after 6 and 12 months of treatment compared with healthy controls. At 12 months after initial presentation, mean steroid dose was tapered to maintenance levels or even withdrawn (6.8 ± 3.0 mg/day). Total leptin levels (26.4 ± 15.0 ng/ml) and total leptinBMI [0.95 ± 0.47 (ng/ml)/(kg/m²)] in patients in long-term remission were not different from healthy controls and vasculitis patients at 6 and 12 months.

Serum ghrelin levels at baseline were significantly elevated in patients compared with healthy controls (402.6 ± 82.2 vs 294.8 ± 70.9 pmol/l; P < 0.005) (Fig. 2). Serum ghrelin levels decreased after treatment reaching statistical significance after 6 months (249.8 ± 112.9 vs 152.9 ± 50.3 pmol/l; P < 0.05).

**Discussion**

Serum leptin levels were significantly lower in patients with AAV when compared with healthy controls. This difference persisted when leptin levels were corrected with BMI. BMI did not change significantly during follow-up. At initial presentation, 38.1% of patients did not report on weight loss, whereas 28.6 and 33.3% reported on weight loss of ≤2 and ≥2 kg, respectively. However, weight loss before initial presentation did not correlate with leptin, leptinBMI or ghrelin. BMI did not change significantly during follow-up. Mean leptin levels increased significantly after 6 (27.8 ± 21.9 ng/ml; P < 0.001) and 12 months (24.6 ± 21.0 ng/ml; P < 0.001) as compared with baseline values, and were no longer different from healthy controls. BMI-corrected leptinBMI also increased significantly after 6 [1.0 ± 0.8 (ng/ml)/(kg/m²); P < 0.001] and 12 months [0.9 ± 0.79 (ng/ml)/(kg/m²); P < 0.001] as compared with baseline values (Fig. 1). Notably, serum levels of leptin and leptinBMI normalized after 6 and 12 months of treatment compared with healthy controls. At 12 months after initial presentation, mean steroid dose was tapered to maintenance levels or even withdrawn (6.8 ± 3.0 mg/day). Total leptin levels (26.4 ± 15.0 ng/ml) and total leptinBMI [0.95 ± 0.47 (ng/ml)/(kg/m²)] in patients in long-term remission were not different from healthy controls and vasculitis patients at 6 and 12 months.

**Table 1. Clinical characteristics of patients with AAV at disease onset, matched healthy controls and patients in long-term remission**

<table>
<thead>
<tr>
<th></th>
<th>Patients with active AAV</th>
<th>Controls</th>
<th>Patients in long-term remission</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of subjects</td>
<td>21</td>
<td>21</td>
<td>16</td>
</tr>
<tr>
<td>Sex (male/female)</td>
<td>8/13</td>
<td>8/13</td>
<td>11/16</td>
</tr>
<tr>
<td>Age (yrs)</td>
<td>55.0 ± 15.9</td>
<td>58.1 ± 15.4</td>
<td>58.2 ± 12.0</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>26.2 ± 3.8</td>
<td>25.8 ± 3.7</td>
<td>27.4 ± 4.8</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>172.6 ± 8.8</td>
<td>171.2 ± 7.9</td>
<td>174.0 ± 9.2</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>78.8 ± 16.5</td>
<td>78.8 ± 14.9</td>
<td>82.2 ± 20.0</td>
</tr>
<tr>
<td>Serum creatinine (µmol/l)</td>
<td>154.4 ± 92.8</td>
<td>81.6 ± 8.5</td>
<td>146.0 ± 82.2*</td>
</tr>
<tr>
<td>GFR (m/l/min)</td>
<td>59.5 ± 32.3</td>
<td>81.7 ± 15.9</td>
<td>62.6 ± 24.9*</td>
</tr>
<tr>
<td>CRP (mg/l)</td>
<td>105.8 ± 103.8</td>
<td>0.9 ± 0.6</td>
<td>2.1 ± 1.7</td>
</tr>
<tr>
<td>CECs (cells/ml)</td>
<td>98.6 ± 160.2**</td>
<td>5.8 ± 3.7</td>
<td></td>
</tr>
<tr>
<td>BVAS</td>
<td>16.0 ± 7.4**</td>
<td>0.0 ± 0.0</td>
<td>0.0 ± 0.0</td>
</tr>
</tbody>
</table>

*p < 0.05 compared with controls. *P < 0.005 compared with controls. **P < 0.001 compared with controls.

**FIG. 1. BMI-corrected serum leptin levels in patients with active AAV at initial presentation (n = 21), during follow-up and in long-term remission (n = 16) compared with matched healthy controls (n = 21). Horizontal bars indicate mean values.**
adjusted for BMI, except that correlation with CEC just missed significance. The same results were obtained when leptin concentrations were compared with baseline values. No differences were seen between controls and patients after 6 months, 12 months and patients in long-term remission.

Circulating ghrelin and leptin concentrations did not correlate with BMI in controls or patients at baseline. There was a highly significant positive correlation between ghrelin and disease activity (CRP: $r_s = 0.500, P < 0.001$; BVAS: $r_s = 0.512, P < 0.001$; CEC: $r_s = 0.338, P = 0.019$), whereas leptin had a negative correlation with disease activity (CRP: $r_s = 0.426, P < 0.005$; BVAS: $r_s = 0.379, P < 0.005$; CEC: $r_s = 0.281, P = 0.044$) when all patients with quiescent and active vasculitis were included. The same results were obtained when leptin concentrations were adjusted for BMI, except that correlation with CEC just missed significance (CRP: $r_s = 0.407, P < 0.005$; BVAS: $r_s = 0.398, P < 0.005$; CEC: $r_s = 0.261, P = 0.061$). Accordingly, correlations between the ghrelin/leptin ratio and markers of disease activity reached the highest level of significance (CRP: $r_s = 0.477, P < 0.001$; BVAS: $r_s = 0.514, P < 0.001$; CEC: $r_s = 0.354, P < 0.001$). Finally, neither leptin nor ghrelin correlated with serum creatinine ($P = 0.700, P = 0.222$) or GFR ($P = 0.714, P = 0.115$) in our patients.

**Discussion**

AAVs are inflammatory disorders which often lead to marked constitutional symptoms and anorexia. It is therefore surprising that leptin and ghrelin levels have not been studied so far. In this study, we detected significantly lower total leptin levels and significantly elevated ghrelin levels in patients with active AAV compared with age- and BMI-matched healthy controls. After 6 and 12 months, both leptin and ghrelin had returned to levels of healthy controls during immunosuppressive therapy and remained stable in long-term remission. Leptin levels displayed a negative correlation with parameters of disease activity (CRP, CECs and BVAS) while a positive correlation with ghrelin was noted. The ghrelin/leptin ratio, which has been described as a key index of the metabolic state, correlated closely with these markers of disease activity.

In contrast to our results, a positive correlation between leptin and inflammation was found in a variety of autoimmune diseases. Serum leptin levels in patients with SLE were elevated although a correlation with disease activity indices was not observed [18]. Notably, elevated serum leptin levels were also detected in Behcet’s disease and a positive correlation with disease activity was seen [19]. One would therefore expect a similar effect in ANCA-associated small-vessel vasculitis. Conversely, a variety of chronic inflammatory diseases, such as tuberculosis, RA or inflammatory bowel disease, all display decreased serum leptin levels [20–22]. In vitro data have demonstrated that long-term stimulation of adipose tissue by pro-inflammatory cytokines such as TNF-α and IL-1β inhibits leptin at the protein and mRNA level [23]. It is difficult to reconcile these contrasting findings but differences in the immunopathogenesis and cytokine profiles may be involved. In this context, it must be appreciated that both lack and excess of leptin may be detrimental as reviewed by Bernotiene and colleagues [5, 6]. High levels of leptin may enhance inflammation in the setting of T-cell-driven autoimmune disease (e.g. RA and multiple sclerosis) [24, 25]. On the other hand, low levels of leptin may enhance the inflammatory process in disorders that involve innate immunity. Accordingly, these disorders may benefit from exogenous leptin supplementation as suggested from various animal models [26, 27]. Unfortunately, there are only limited data regarding the involvement of innate immunity in AAV while T cells are clearly involved during the pathogenesis [28].

A positive correlation between elevated ghrelin and TNF-α levels has been described in cachexia [20, 29]. We detected elevated serum ghrelin levels in our vasculitis patients at presentation. Elevated ghrelin levels are present in chronic renal failure although the underlying mechanism remains controversial [30]. However, since active AAV might initially cause acute renal failure, a comparison with reports on chronic renal failure might be misleading. None of our patients required haemodialysis due to acute renal failure. Serum levels of ghrelin in acute renal failure have not been investigated so far. However, we did not detect a correlation between ghrelin levels and renal function in the present study.

Ghrelin has been shown to attenuate leptin-induced pro-inflammatory responses in human mononuclear and T cells [10]. In the present study, the ghrelin/leptin ratio correlated particularly well with disease activity. It is conceivable to use this ratio to characterize the state of the ghrelin/leptin system at presentation and during follow-up. It would be interesting to see whether the ghrelin/leptin ratio changes just before relapse or after infectious complications.

Invasion and destruction of activated endothelium by primed neutrophils is a salient feature of vasculitis. Notably, ghrelin and leptin may partake in this process because microvascular endothelial cells and neutrophils both express the leptin and ghrelin receptor [31–34]. Further evidence has emerged from a study by Ottonello et al. [31] who demonstrated a marked inhibitory effect of leptin on leucocyte chemotaxis. Furthermore, leptin administration to leptin-deficient ob/ob mice has been demonstrated to blunt the neutrophil influx into skin wounds [35]. It is thus conceivable that leptin attenuates the neutrophil chemotaxis in vasculitis. Further effects of leptin on microvascular endothelial cells include increased proliferation and inhibition of apoptosis [32]. Effects of ghrelin on endothelial cells have also been described. Ghrelin has been shown to increase expression of adhesion molecules [36] and to exhibit anti-proliferative effects on microvascular endothelial cells [33, 34]. It is conceivable that leptin, ghrelin, or both, exert direct effects on endothelial cells
Regarding the pathogenesis of vasculitis and such effects clearly deserve further study.

It is well established that ghrelin and leptin link nutritional status and the immune response [4, 37]. Our finding of low leptin levels in patients at presentation may relate to the susceptibility to infections that frequently trigger the disease [38]. Notably, models of genetic leptin deficiency have shown that leptin supplementation reverses susceptibility to infections [39, 40]. Surprisingly, weight loss before initial presentation did not correlate with leptin, leptin/ghrelin or ghrelin in the present study. Thus, weight loss in AAV might be mediated by other regulatory mechanisms.

In conclusion, we found decreased serum levels of leptin and increased serum levels of ghrelin in active vasculitis. Leptin and ghrelin returned to normal during immunosuppressive therapy and remained stable in long-term remission. The role of the ghrelin/leptin system in AAV deserves further study, not least because perturbations in this system may affect endothelial cells and thus contribute to disease pathogenesis. Animal models of AAV have become available and the course of disease in leptin-deficient animals would be of particular interest.

### Rheumatology key messages

- Active AAV is characterized by decreased serum leptin and increased serum ghrelin, both of which return to normal with successful therapy.
- Leptin, ghrelin and ghrelin/leptin ratio correlate with disease activity in AAV.
- Decreased leptin in active AAV might account for susceptibility to concomitant bacterial infections.

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### Disclosure statement

The authors have declared no conflicts of interest.

### References


