Associations between plasma ghrelin levels and body composition in end-stage renal disease: a longitudinal study

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Abstract

Background. Ghrelin is a newly detected orexigenic gastric hormone that stimulates food intake. Increased levels of ghrelin are often found in disease states associated with wasting. Wasting is a common phenomenon in end-stage renal disease (ESRD) patients in whom elevated ghrelin levels have been reported. However, no data are available on the relationship between body composition and plasma ghrelin levels in this patient group.

Methods. The study population consisted of 108 (71 males) ESRD patients aged 53±12 years. Body composition, nutritional status (subjective global assessment), estimated protein intake (nPNA), plasma ghrelin, plasma insulin and serum leptin were evaluated close to the start of dialysis treatment. Twelve healthy subjects (nine males, 44±6 years) served as the control group. A longitudinal evaluation of changes in plasma ghrelin and body composition was performed in 52 of the patients after 12 months of dialysis treatment.

Results. Markedly elevated plasma ghrelin levels (843±485 vs 443±302 pg/ml; P<0.01) were observed in ESRD patients compared with controls. Basal plasma ghrelin levels correlated significantly with plasma insulin (R=-0.32; P<0.05), body mass index (R=-0.24; P<0.05), log serum leptin levels (R=-0.23; P<0.05) and truncal fat mass (R=-0.25; P<0.05). The longitudinal analysis of body composition demonstrated that whereas fat mass increased (23.7±8.6 to 25.3±9.9 kg; P<0.05) and plasma ghrelin levels decreased (855±429 to 693±408 pg/ml; P<0.05) significantly in peritoneal dialysis patients, no significant changes in either body composition or plasma ghrelin levels were found in patients treated by haemodialysis.

Conclusion. Markedly elevated plasma ghrelin levels are found in advanced renal failure and correlate with fat mass, plasma insulin and serum leptin levels. Changes in plasma ghrelin during 12 months of peritoneal dialysis treatment are associated with changes in body composition.

Keywords: body composition; end-stage renal disease; ghrelin; insulin; leptin; peritoneal dialysis

Introduction

Protein-energy malnutrition and wasting, which are common phenomena in patients with end-stage renal disease (ESRD), are associated with an increased morbidity and mortality [1]. Several factors may contribute to malnutrition in ESRD, in particular inflammation, co-morbidity and anorexia caused by uraemic toxicity. The mechanism(s) causing decreased appetite in uraemia are not fully understood. Leptin (the product of the ob gene) has attracted considerable interest as a putative factor regulating appetite in ESRD patients [2]. Although a few studies suggest that hyperleptinaemia may play a role in anorexia [3], loss of body weight [4] and lean body mass [5] in ESRD patients, others [6] find no association between leptin and recent changes in weight, body composition or nutritional status in ESRD patients. Thus, as available data do not clearly support a pivotal role for leptin in body weight regulation in ESRD, other critical pathways need to be elucidated. Recent interest has therefore focused on ghrelin, a new hormone, which may have a function in the orexigenic pathway downstream from leptin [7].

Ghrelin, first described by Kojima et al. [8], is a peptide of 28 amino acids (3315 Da) that stimulates growth hormone release from the pituitary [7]. Ghrelin is secreted into the bloodstream primarily from

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endocrine cells within the stomach [7]. However, recent evidence suggests that other tissues also synthesize ghrelin, including the kidney [9]. Ghrelin has been reported to regulate feeding and body weight regulation through stimulation of hypothalamic appetite centres [10] and coordination of energy balance [11]. Although its initial discovery was as a novel growth hormone secretagogue, it has been found to regulate feeding behaviour by modulating expression levels of orexigenic peptides in the hypothalamus. Ghrelin has been implicated in the coordination of energy balance and weight regulation, and its dysregulation may be important in obesity. It should be pointed out that ghrelin also has several other physiological actions besides the potential regulation of food intake [11]. Recent studies demonstrated that ghrelin antagonizes leptin action and promotes the production of orexigenic neuropeptides, such as neuropeptide Y, resulting in an increase in feeding and body weight [12]. Indeed, peripheral infusion of ghrelin stimulates food intake in both rats [12] and humans [13]. In general, loss of body fat mass and wasting due to cancer [14], cardiac cachexia [15] or anorexia nervosa [16] is associated with elevated circulating levels of ghrelin. Surprisingly, circulating ghrelin levels seem to be downregulated in human obesity, and negative correlations between ghrelin and both serum leptin and plasma insulin have been reported [17]. Thus, it is unlikely that overproduction of ghrelin causes the obesity syndrome. Although Yoshimoto et al. [18] reported 2.8-fold higher ghrelin and desacyl ghrelin levels in patients with renal failure, data on the association between ghrelin levels and body composition in ESRD patients have, to the best of our knowledge, not been reported in the literature. As fasting ghrelin concentrations are suppressed by feeding and oral glucose administration [10], we hypothesize that during peritoneal dialysis (PD), the continuous glucose absorption may result in an increase in feeding and body weight [12]. Indeed, peripheral infusion of ghrelin stimulates food intake in both rats [12] and humans [13]. In general, loss of body fat mass and wasting due to cancer [14], cardiac cachexia [15] or anorexia nervosa [16] is associated with elevated circulating levels of ghrelin. Surprisingly, circulating ghrelin levels seem to be downregulated in human obesity, and negative correlations between ghrelin and both serum leptin and plasma insulin have been reported [17]. Thus, it is unlikely that overproduction of ghrelin causes the obesity syndrome. Although Yoshimoto et al. [18] reported 2.8-fold higher ghrelin and desacyl ghrelin levels in patients with renal failure, data on the association between ghrelin levels and body composition in ESRD patients have, to the best of our knowledge, not been reported in the literature. As fasting ghrelin concentrations are suppressed by feeding and oral glucose administration [10], we hypothesize that during peritoneal dialysis (PD), the continuous glucose absorption may result in changes in body composition which in turn may affect circulating ghrelin concentrations.

The purpose of the present study was therefore (i) to determine plasma concentrations of ghrelin in a group of patients with ESRD at the start of renal replacement therapy; and (ii) in a longitudinal study, relate changes in plasma ghrelin levels to changes in body composition in patients who had completed 12 months of dialysis treatment.

Subjects and methods

Subjects

A total of 108 ESRD patients (71 males; mean age 53 ± 12 years) were studied close (35 ± 106 days) to the start of dialysis treatment. They were selected from an ongoing prospective study, and part of this patient material has been described previously [19]. The study exclusion criteria were age > 70 years, overt infectious complication and unwillingness to participate in the study. Seventy-three of the patients were non-diabetics, whereas 15 patients had type 1 and 20 patients had type 2 diabetes mellitus. Most patients were on antihypertensive medications as well as other commonly used drugs in ESRD, such as phosphate and potassium binders, diuretics and vitamin B, C and D supplementation. The control group consisted of 12 healthy controls (nine males; mean age 44 ± 6 years) with a body mass index (BMI) of 24.5 ± 3.5 kg/m². The Ethics Committee of the Karolinska Institutet at Huddinge University Hospital, Stockholm approved the study protocol, and informed consent was obtained from all subjects.

Blood sampling and laboratory analyses

After an overnight fast, plasma samples were taken in the morning at a similar baseline time and stored at −70°C until analysed. Glomerular filtration rate (GFR) was estimated by the mean of urea and creatinine clearance in a 24 h urine collection. Nutritional status (n = 100) was recorded on the same occasion using subjective global assessment (SGA), as previously described [19]. Patients scored as having SGA > 1 were considered to be malnourished. Determinations of serum albumin ( bromcresol purple) and serum creatinine, as well as urinary excretion of creatinine and urea, were performed by routine procedures at the Department of Clinical Chemistry, Huddinge University Hospital. Serum leptin levels (n = 98) were analysed with a commercially available radioimmunoassay (RIA) kit (Linco Research Inc., St Charles, MO). A specific RIA assay (Pharmacia, Uppsala, Sweden) was used to analyse plasma insulin (n = 55). Plasma ghrelin was measured by an RIA method (Phoenix Pharmaceuticals Inc. Belmont, CA). The sensitivity was 1.2 pg/ml. Ninety-two of the patients had their body composition evaluated by dual-energy X-ray absorptiometry (DXA) (Lunar Corp., Madison, WI) with Lunar software version 3.4.

To study the impact of dialysis treatment on body composition and plasma ghrelin levels, a longitudinal follow-up was performed in 52 of the 108 patients (34 males; mean age 54 ± 12 years), following 12 months of dialysis treatment. Thirty-three of the patients started PD, whereas 19 patients started haemodialysis (HD). Routine bicarbonate HD was carried out three times a week (4–5 h per session) using standard cellulose acetate or polysulfone dialysis membranes. Dietary recommendations were given routinely to maintain a daily protein intake exceeding 1.0 g/kg body weight in both groups of dialysis patients. Protein intake was monitored by urea kinetics whereas energy intake was not routinely monitored. Determinations of fasting plasma for ghrelin and body composition (DXA) were performed again 12 months after the patients had started dialysis treatment.

Statistical analysis

Results are expressed as mean values ± SD, with P < 0.05 indicating significance. As serum leptin was non-normally distributed, it was log10 transformed before entering any statistical analysis. Longitudinal comparisons were made by the paired t-test, whereas comparisons between two groups were made by unpaired Student’s t-test. Correlations were performed by linear regression analysis. The statistical analysis was performed using Statview® version 5.00 for Windows (SAS Institute, Berkley, CA).
Results

The clinical and biochemical characteristics of the ESRD patients are given in the Table 1. Plasma levels of ghrelin were markedly higher in ESRD patients than controls ($843\pm485$ vs $443\pm302$ pg/ml; $P<0.01$), as shown in Figure 1. No significant differences in plasma ghrelin levels were observed between non-diabetics ($874\pm483$ pg/ml), type 1 ($801\pm546$ pg/ml) and type 2 ($758\pm459$ pg/ml) diabetic patients, respectively. Thirty-one patients grouped as malnourished (SGA $>1$) had ghrelin levels similar ($826\pm423$ pg/ml) to those of 69 well-nourished patients. Thirty-seven female ESRD patients ($984\pm533$ pg/ml) had significantly ($P<0.05$) higher ghrelin levels than 71 males ($769\pm444$ pg/ml). A significant inverse correlation was observed between plasma ghrelin and plasma insulin ($R=-0.32; P<0.05; n=55$) (Figure 2). When diabetic and non-diabetic patients were analysed separately, a significant correlation between plasma insulin and plasma ghrelin was found in non-diabetic patients only ($R=-0.47; P<0.01; n=42$). As expected, an inverse correlation between plasma ghrelin and log serum leptin ($R=-0.23; P<0.05; n=98$) was observed. Whereas significant inverse correlations were observed between fasting ghrelin and BMI ($R=-0.24; P<0.05$) and truncal fat mass ($R=-0.25; P<0.05$), the correlation between ghrelin and total fat mass ($R=-0.19; P=0.07$) did not reach statistical significance. No significant correlations were observed between ghrelin and age ($R=0.08$), GFR ($R=0.04$), estimated protein intake ($R=0.03$) or lean body mass ($R=0.16$).

Data on body composition and plasma ghrelin levels in the 52 ESRD patients that were evaluated following 12 months of RRT are given in Table 2. The repetitive analysis of body composition with DXA demonstrated that whereas lean body mass decreased and both total fat mass and truncal fat mass increased significantly in PD patients, no significant changes in the body composition were noted among patients treated by HD. Similarly, whereas no changes in plasma ghrelin levels were found in HD patients, plasma ghrelin decreased significantly ($P<0.05$) from $855\pm429$ to $693\pm408$ pg/ml in PD patients. In the combined patient group, significant correlations were observed between changes (Δ) in plasma ghrelin and Δ total body mass ($R=0.45; P<0.01$) as well as Δ total ($R=-0.45; P<0.01$) and Δ truncal ($R=-0.40; P<0.01$) fat mass, respectively (Figure 3). Whereas significant correlations were found between Δ plasma ghrelin and Δ total fat mass ($R=-0.56; P<0.01$), Δ

Table 1. Clinical data and body composition in 108 ESRD patients at the start of renal replacement therapy

| Male gender (%) | 66 |
| GFR (ml/min) | 6.7±2.1 |
| Malnutrition (SGA >1) (%) | 31 |
| Body mass index (kg/m²) | 25.1±4.4 |
| Lean body mass (kg) | 50.2±9.7 |
| Body fat mass (kg) | 21.7±8.7 |
| Truncal fat mass (kg) | 11.6±5.2 |
| Protein intake (g/kg) | 0.72±0.16 |
| Plasma insulin (mU/L) | 26.5±14.0 |
| Serum leptin (ng/ml) | 11.3±195.0 |
| Plasma ghrelin (pg/ml) | 843±485 |

$^a n=100; ^b n=92; ^c n=75; ^d n=55; ^e n=98; ^f$ median and range.

Fig. 1. Box plot showing a significant difference ($P<0.01$) in basal fasting plasma ghrelin levels between healthy controls and ESRD patients.

Fig. 2. Correlations between fasting plasma ghrelin and plasma insulin levels in non-diabetic (filled circles) and diabetic (open circles) ESRD patients.

Table 2. Changes in body composition and plasma ghrelin levels in patients treated by haemodialysis or peritoneal dialysis during 12 months

<table>
<thead>
<tr>
<th>Haemodialysis (n = 19)</th>
<th>basal</th>
<th>12 months</th>
<th>significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>BMI (kg/m²)</td>
<td>24.1±3.9</td>
<td>23.5±3.5</td>
<td>NS</td>
</tr>
<tr>
<td>Lean body mass (kg)</td>
<td>52.9±10.3</td>
<td>51.4±10.9</td>
<td>NS</td>
</tr>
<tr>
<td>Body fat mass (kg)</td>
<td>19.8±8.7</td>
<td>19.4±8.7</td>
<td>NS</td>
</tr>
<tr>
<td>Truncal fat mass (kg)</td>
<td>11.0±5.2</td>
<td>10.8±5.3</td>
<td>NS</td>
</tr>
<tr>
<td>Plasma ghrelin (pg/ml)</td>
<td>853±514</td>
<td>812±484</td>
<td>NS</td>
</tr>
</tbody>
</table>

| Peritoneal dialysis (n = 33) |
|-----------------------------|-------|-----------|--------------|
| BMI (kg/m²) | 25.8±3.5 | 25.9±4.0 | NS |
| Lean body mass (kg) | 49.7±8.8 | 48.8±8.4 | $P<0.001$ |
| Body fat mass (kg) | 23.7±8.6 | 25.3±9.9 | $P<0.01$ |
| Truncal fat mass (kg) | 12.6±4.0 | 13.4±4.9 | $P<0.01$ |
| Plasma ghrelin (pg/ml) | 855±429 | 693±408 | $P<0.05$ |

$^a n=18; ^b n=26.$
truncal fat mass \((R = -0.52; P < 0.01)\) and \(\Delta\) lean body mass \((R = 0.43; P < 0.05)\) in PD patients, no significant correlations between \(\Delta\) plasma ghrelin and either \(\Delta\) total fat mass \((R = -0.12)\), \(\Delta\) truncal fat mass \((R = -0.13)\) or \(\Delta\) lean body mass \((R = 0.38)\) were found in HD patients.

**Discussion**

The present study demonstrates markedly elevated plasma ghrelin levels in ESRD patients close to the start of dialysis treatment (Figure 1). Thus, our results correspond to recent findings by Yoshimoto et al. [18] suggesting that the kidney is an important site for clearance and/or degradation of ghrelin. In the present study, no significant correlation between GFR and ghrelin was found. However, as all included patients had advanced renal failure, the narrow range of GFR (3–16 ml/min) may explain the lack of a significant correlation. It could be speculated that the increased plasma ghrelin concentration observed in the present study may rather represent a physiological adaptation to a long-term negative energy balance associated with wasting in ESRD patients. Indeed, Masaoka et al. [20] recently demonstrated in streptozotocin-induced diabetes in rats that a negative energy balance may enhance ghrelin secretion into the bloodstream. However, as we found no difference in plasma ghrelin levels between ESRD patients with and without signs of wasting (as indicated by SGA), respectively, we find this hypothesis unlikely in this particular clinical setting. Moreover, although ghrelin is supposed to stimulate food intake, no association was found between plasma ghrelin levels and estimated protein intake (nPNA) in the present study.

The observed negative correlations between plasma ghrelin and BMI and visceral (truncal) fat mass in the present study are in agreement with previous findings in non-renal patient groups, demonstrating that ghrelin is downregulated in obesity [17]. Moreover, the association between plasma ghrelin and body composition in ESRD is indirectly supported by significant inverse correlations between ghrelin and both plasma insulin (Figure 2) and serum leptin. Indeed, recent findings by Saad et al. [21] indicate that insulin is a physiological and dynamic modulator of plasma ghrelin. On the other hand, a recent study demonstrated that a reduction in ghrelin is only seen at supraphysiological insulin concentrations [22]. Also, leptin, which is independently associated with hyperinsulinaemia in ESRD [23], has been shown to be inversely related to ghrelin in other patient groups [17].

The main finding of the present study was that 12 months of PD was associated with a significant reduction of plasma ghrelin levels, whereas no changes in plasma ghrelin levels were observed in patients treated by HD. Moreover, in PD patients, a significant correlation was observed between changes in fat mass and plasma ghrelin levels (Figure 3). There may be several reasons why plasma ghrelin levels decrease during 12 months of PD. Firstly, as the observed decrease in the plasma ghrelin concentration during PD was significantly linked to increased fat mass, changes in body composition may be one contributing factor. Indeed, our results are in accordance with a previous finding in patients with anorexia nervosa showing that increased plasma ghrelin concentrations normalized following weight recovery [16]. However, the possibility of downregulation of the ghrelin system by increased fat mass should not be overemphasized, as a different pattern might be present in ESRD patients as renal function seems to affect plasma ghrelin levels so much. Therefore, we find it likely that hyperglycaemia and hyperinsulinaemia, two common findings in PD patients, may also be related to the observed decrease in plasma ghrelin levels during PD. Clearly, studies are needed to elucidate the effect of intraperitoneal glucose absorption on gastric ghrelin secretion in PD patients. It could also be speculated that the observed differences in plasma ghrelin levels during HD and PD may be related to differences in changes of residual renal function. However, as plasma ghrelin levels remained unchanged (HD) or decreased (PD), a fall in residual renal function is considered an unlikely cause of the observed changes in plasma ghrelin levels.
Ghrelin in ESRD
gールin during dialysis treatment. Finally, it may be speculated that peritoneal clearance during PD may contribute to the observed decrease of plasma ghrelin levels. However, considering the molecular weight of ghrelin (3.3 kDa), the calculated transperitoneal solute clearance may be negligible (2–3 ml/min).

Clearly, further studies are needed to investigate if decreasing ghrelin concentrations may be one reason for the low eating drive despite a need for protein and calories documented in PD patients [24]. Further research is also needed to investigate whether or not ghrelin resistance with impairment of intracellular ghrelin receptor signalling are present in ESRD patients. It is interesting to note that whereas the plasma levels of ghrelin are high and the plasma levels of leptin are low in wasted ESRD patients, the opposite is observed in obese ESRD patients. In fact, it seems like the ghrelin and leptin systems are affected in opposite directions, perhaps reflecting failed attempts to modify appetite and correct the nutritional abnormalities in ESRD patients. However, both the leptin and ghrelin systems appear to be insufficient in this respect, and other mechanisms may therefore be more important for regulation of appetite and nutritional status in patients with ESRD.

Some shortcomings of the present study should be discussed. First, although ghrelin has been identified as a ligand for growth hormone, this hormone was not assessed in the present study. Secondly, although DXA has been shown to be superior to other simple non-invasive methods to determine body composition in ESRD, it should be noted that the estimation of lean body mass may be confounded by changes in hydration status. However, we have demonstrated previously that changes in hydration status could not explain changes in lean body mass over time [5]. Another point of criticism might be that we used an RIA method that analysed the sum of both ghrelin and desacyl ghrelin, a presumably inactive metabolite which may account for >90% of total circulating ghrelin [18]. As the study by Yoshimoto et al. [18] reported that another RIA test (not commercially available) that represents ghrelin alone did not correlate with renal function, it seems conceivable that accumulation of the inactive metabolite desacyl ghrelin accounts for the major part of the observed increase in plasma ghrelin concentration in ESRD. Thus, as desacyl ghrelin does not appear to possess metabolic activities, further studies are needed to evaluate the role of ghrelin as such in ESRD. Finally, it should be pointed out that this is a post hoc analysis, which may limit the value of the study.

In conclusion, the present study demonstrates markedly elevated plasma concentrations of ghrelin in advanced renal failure and that increased body fat mass during PD is related to changes in plasma ghrelin concentrations. Further studies are needed to examine relative changes in ghrelin vs inactive ghrelin metabolites during the course of progression of renal failure and the putative associations with changes in food intake and body composition.

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Conflict of interest statement. B. Lindholm is an employee of Baxter Healthcare Inc.

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