Plasma ghrelin levels in patients undergoing haemodialysis and peritoneal dialysis

Miguel Pérez-Fontán1,4, Fernando Cordido2,4, Ana Rodríguez-Carmona1, Javier Peteiro3, Rafael García-Naveiro1 and Jesús García-Buela3

1Division of Nephrology, 2Division of Endocrinology and 3Division of Laboratory, Hospital Juan Canalejo, A Coruña, Spain and 4Department of Medicine, Health Sciences Institute, University of A Coruña, Spain

Abstract

Background. Ghrelin has been characterized as a relevant physiologic regulator of appetite and body weight in humans. However, the potential relationships between ghrelin levels, inflammation and malnutrition in dialysis patients have not been adequately studied.

Methods. We used a cross-sectional design to study 20 haemodialysis (HD) and 21 peritoneal dialysis (PD) patients, and compared their plasma ghrelin (PGhr) levels with that of an age-matched control group. We also explored correlations between ghrelin and selected hormonal, renal adequacy, nutritional and inflammation markers in both groups.

Results. PGhr levels were higher in HD (median 119.8 pg/ml, range 71.1–333.7, P = 0.001) and PD (99.3, range 45.8–578.5, P = 0.045) patients than in healthy controls (78, range 29–158) (HD vs PD, not significant). Ghrelin levels were strongly and inversely correlated with age (r = −0.46, P = 0.02 for patients; r = −0.61, P = 0.001 for controls). Except for a positive correlation between ghrelin and growth hormone (r = 0.48, P = 0.002), univariate analysis failed to detect associations between PGhr and the measured hormonal values, renal adequacy, nutritional indicators and markers of inflammation. However, multivariate analysis revealed significant inverse correlations between PGhr levels and nutritional markers, including subjective global assessment (P = 0.013), albumin (P = 0.001), transferrin (P = 0.01) and protein nitrogen appearance (as an estimate of protein intake) (P = 0.035), after controlling for the confounding effect of age.

Conclusions. PGhr levels were moderately and similarly increased in patients undergoing HD and PD. Age was a strong determinant of PGhr levels, both in uraemic patients and in healthy controls. Dialysis adequacy, residual renal function and inflammation did not appear to influence ghrelin levels in these patients. The negative correlation between PGhr and nutritional markers suggests that low dietary intake causes increases in ghrelin secretion in dialysis patients.

Keywords: ghrelin; haemodialysis; inflammation; malnutrition; peritoneal dialysis

Introduction

Ghrelin is a post-translationally modified (octanoylated) 28 amino acid peptide that is secreted predominantly, but not exclusively, by the X/A oxyntic cells of the stomach [1,2]. Initially characterized as an endogenous growth hormone (GH) secretagogue [1], there is now strong evidence that ghrelin plays a prominent role in the physiologic regulation of appetite and body weight [3]. By acting as a potent blood-borne orexigenic signal from the gut to the brain [4], ghrelin stimulates spontaneous food intake and induces fat and weight gain by GH-independent mechanisms. Plasma ghrelin (PGhr) levels are strongly influenced by oral intake and peak during fasting but decrease rapidly after meals [3].

At present, there is little information on the behaviour of ghrelin in patients with chronic renal failure. A recent study examining 41 patients having varying degrees of renal insufficiency found increased plasma levels of C-terminal but not N-terminal ghrelin [5]. However, this study included few patients undergoing dialysis therapy, and all were treated with haemodialysis (HD). Moreover, this work failed to explore potential correlations between PGhr and markers of malnutrition and inflammation. Anorexia and malnutrition are common in uraemic patients presenting with increased inflammatory activity, and this association may be mediated in part by increased plasma levels of leptin, a powerful inhibitor of appetite [6]. Although it
is additionally possible that inflammation may affect ghrelin secretion to thereby stimulate anorexia in these patients, this mechanism has not yet been investigated. As a further unexplored mechanism, intraperitoneal glucose infused during peritoneal dialysis (PD) therapy may directly or indirectly affect ghrelin levels through an effect on insulin secretion.

The present cross-sectional study aimed at comparing PGhr levels in patients treated with HD and PD, and examined potential correlations between this peptide and selected markers of inflammation and malnutrition.

Patients and methods

Following a cross-sectional design, we studied 41 randomly selected, stable uremic patients undergoing HD (n = 20) or PD (n = 21). The main characteristics of the study sample are presented in Table 1. The two groups were comparable, except for a slight but non-significant greater prevalence of diabetes in the PD group. No patients had type I diabetes or had undergone renal transplantation before the study. PGhr levels were also determined in a group of 26 healthy controls that were matched for age (Table 1). Following an overnight fast, blood samples were collected between 09.00 and 09.30 randomly between HD and PD patients and controls, except that samples were always obtained on non-dialysis days in HD patients. In PD patients, blood was collected after the first daytime exchange, using 1.36% glucose or icodextrin (automated PD) dialysate in all cases. Blood samples for PGhr determination were collected in chilled tubes containing EDTA-Na and aprotinin, were centrifugated immediately at 4°C, and were frozen at −80°C for a maximum of 3 weeks before processing. PGhr levels were measured using a standard radioimmunoassay that detects full-length octanoylated human ghrelin (Phoenix Pharmaceuticals, Belmont, CA, USA).

Plasma albumin, prealbumin, transferrin, cholesterol and triglycerides, as well as haemoglobin and lymphocyte counts, were determined by an autoanalyser. We also recorded dry body weight, body mass index (BMI) (body weight/height²), and protein nitrogen appearance (normalized for actual body weight) as a correlate of daily protein intake (urea kinetics) [7,8]. Finally, standard subjective global assessment (SGA) [9] was carried out in all patients.

To assess renal function and dialysis adequacy, we measured 24 h diuresis, mean renal clearance [urea clearance + creatinine clearance/2], total Kt/V [7,8], 24 h proteinuria and 24 h peritoneal protein losses (PD).

To determine the degree of inflammation, we measured C-reactive protein (high sensitivity assay) (Immunoturbidimetry; Roche Diagnostics, Mannheim, Germany), interleukin-6 (ELISA; R&D Systems, Minneapolis, MN, USA), tumour necrosis factor-α (TNF-α) (ELISA; R&D), intercellular adhesion molecule (ICAM) (ELISA; R&D) and vascular adhesion molecule (VCAM) (ELISA; R&D).

We compared PGhr in patients treated with HD and PD, and in healthy controls. In HD and PD patients, we also examined potential correlations between PGhr and hormonal, nutritional, renal adequacy and inflammatory markers. Numerical variables are presented as median values (range). Statistical analysis was performed using non-parametric tests. Numerical variables were compared by Mann–Whitney tests and the Spearman correlation coefficient. Categorical variables were compared by χ² analysis. We also applied multiple regression analysis to adjust for age in any of the potential correlations between PGhr and the main variables. We used the SPSS 11.5 software for these analyses.

Results

General description

PGhr was significantly higher in both HD (P = 0.001) and PD (P = 0.045) patients than in healthy controls (Figure 1), but was not different between the two dialysis groups (P = 0.25) (Mann–Whitney).

Tables 2 and 3 depict the hormonal, renal adequacy and nutritional data, as well as the inflammatory markers, in both groups. Six patients (four HD, two PD) were malnourished according to SGA, but only one PD patient was classified as severely malnourished.
part of the analysis was carried out with the entire group of dialysis patients \( (n = 41) \). This strategy did not modify the significance of the univariate correlations between ghrelin and renal adequacy, hormonal and inflammation variables displayed in Table 4. However, the multivariate analysis revealed a clear trend for an inverse correlation between PGhr and nutritional markers after controlling for age (Table 5).

**Analysis of subgroups**

The six malnourished patients (according to SGA) tended to be older (median 71 vs 65 years, \( P = 0.11 \)) had higher levels of GH (5.5 vs 2.3 ng/mL, \( P = 0.05 \)), and lower levels of BMI (20.9 vs 25.7 kg/m\(^2\), \( P = 0.02 \)), leptin (5.1 vs 32.8 ng/mL, \( P = 0.006 \)) and insulin (20.5 vs 27.0 mcU/mL, \( P = 0.02 \)) than well nourished patients. PGhr levels did not significantly differ between malnourished (92.4 pg/mL, range 71.1–578.5) and well nourished patients (119.9 pg/mL, range 45.8–333.7) \( (P = 0.79) \).

According to the C-reactive protein values, 18 patients (43.9%) had slight inflammation (5–10 mg/l), and 10 patients (24.3%) had marked inflammation (>10 mg/l). C-reactive protein was strongly correlated with interleukin-6 \( (r = 0.59, P < 0.001) \), but not with TNF-\( \alpha \), ICAM or VCAM. Patients with marked inflammation tended to be older (66 vs 60 years, \( P = 0.24 \)) and presented slightly (not significant) worse nutritional markers than patients without inflammation. PGhr levels were similar in the three subcategories of C-reactive protein levels (medians of 126.3, 117.7 and 113.1 pg/mL in patients with markedly, slightly and no inflammation, respectively, \( P = 0.81, Kruskall–Wallis)\).

Diabetic patients \( (n = 9) \) had higher insulinaemia (median 47.3 vs 25.0 mcU/mL, \( P = 0.03 \)) and BMI (30.9 vs 24.3 kg/m\(^2\), \( P = 0.014 \)), and lower GH levels (1.1 vs 4.6 ng/mL, \( P = 0.035 \)) than non-diabetic patients. PGhr were similar in diabetic (125.1 pg/mL, range 45.8–323.5) and non-diabetic (110.4 pg/mL, range 63.1–578.5) patients \( (P = 0.91) \). PGhr and insulinaemia were poorly correlated in both diabetic and non-diabetic patients (Table 4).

**Discussion**

Many studies have demonstrated a relevant role for ghrelin in the regulation of appetite and body weight. The orexigenic effect of this peptide was first described in an animal model [10]. Since then, other studies have shown that ghrelin is strongly implicated in the day-to-day regulation of appetite and in the long-term control of body weight [11]. The best evidence for the clinical relevance of circulating ghrelin in humans was provided by Cummings et al. [12], who investigated PGhr levels after diet-induced weight loss or gastric bypass surgery. The increase in PGhr observed after diet-induced weight loss lends strong support to the

![Fig. 1. Box plot diagrams of PGhr levels in patients and controls (\( P = 0.045, PD \) vs controls; \( P = 0.001, HD \) vs controls; not significant, PD vs HD) (Mann–Whitney).](https://academic.oup.com/ndt/article-abstract/19/8/2095/1918356)
hypothesis that ghrelin plays a relevant role in the long-term regulation of body weight. This study also showed that gastric bypass surgery produced a marked reduction of PGhr levels, which may have contributed to the long-term weight reducing effect of this procedure.

In the current study, we found that HD and PD patients had moderately but significantly increased levels of total PGhr compared with normal controls. Although Yoshimoto et al. [5] found markedly increased ghrelin levels in 41 patients with renal failure (10 with terminal renal failure, all treated with HD); however, this increase corresponded to essentially C-terminal ghrelin (including desacylated, physiologically inactive ghrelin), whereas the concentration of the N-terminal fraction (acylated ghrelin) did not differ markedly from normal controls. The same study [5] demonstrated significant urinary excretion of ghrelin, as well as significant removal of both the C-terminal and N-terminal fractions by HD.

The mode of dialysis may influence PGhr levels by at least two mechanisms. First, obesity is more prevalent in PD than in HD patients, and PGhr is known to be lower in obese patients than in normal controls [3]. In addition, the intraperitoneal glucose given during PD may inhibit ghrelin secretion since intravenous glucose has been shown to reduce PGhr levels [13], although others failed to obtain this effect [14,15]. The glucose-induced hyperinsulinaemia during dialysis may also contribute to inhibit ghrelin secretion in PD patients [13,16]. Together, these factors may contribute to the high plasma leptin levels observed in PD patients compared with HD patients [17]. Despite these observations, we observed similar PGhr levels under both modes of renal replacement therapy (Figure 1). However, neither BMI nor insulin levels were different between the two groups in our study. Moreover, we want to emphasize that PGhr levels were estimated under specific conditions (see Patients and Methods), and a short-term effect of hypertonic glucose dialysate on PGhr levels cannot be excluded from our data.

Interestingly, we obtained low mean absolute levels of PGhr in both patients and controls. As a whole, the reported reference values for PGhr are remarkably variable. To explain this, different methods of estimation of PGhr may render markedly different results, making it difficult to compare the absolute values reported by Yoshimoto et al. [5] with the present results. In addition, even studies using the same method have reported a wide spectrum of normal reference

<table>
<thead>
<tr>
<th>Table 3. Renal adequacy, nutritional and inflammatory markers</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>Haemodialysis</td>
</tr>
<tr>
<td>----------------</td>
</tr>
<tr>
<td>Diuresis (ml/day)</td>
</tr>
<tr>
<td>Mean renal clearance (ml/min)</td>
</tr>
<tr>
<td>Total Kt/V</td>
</tr>
<tr>
<td>Proteinuria (g/day)</td>
</tr>
<tr>
<td>Peritoneal protein losses (g/day)</td>
</tr>
<tr>
<td>Malnutrition by SGA (% normal/moderate/severe)</td>
</tr>
<tr>
<td>Albumin (g/l)</td>
</tr>
<tr>
<td>Prealbumin (mg/dl)</td>
</tr>
<tr>
<td>Transferrin (mg/dl)</td>
</tr>
<tr>
<td>Cholesterol (mg/dl)</td>
</tr>
<tr>
<td>Triglycerides (mg/dl)</td>
</tr>
<tr>
<td>Haemoglobin (g/dl)</td>
</tr>
<tr>
<td>Lymphocyte count (/mm³)</td>
</tr>
<tr>
<td>Body weight (kg)</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
</tr>
<tr>
<td>nPNA (g/kg/day)</td>
</tr>
<tr>
<td>C-reactive protein (mg/l)</td>
</tr>
<tr>
<td>Interleukin-6 (pg/ml)</td>
</tr>
<tr>
<td>TNF-α (pg/ml)</td>
</tr>
<tr>
<td>ICAM (ng/ml)</td>
</tr>
<tr>
<td>VCAM (ng/ml)</td>
</tr>
</tbody>
</table>

Figures denote median values, except SGA, with range in parentheses. Comparisons by Mann–Whitney tests and χ² analysis. nPNA, normalized protein nitrogen appearance rate.

Fig. 2. Correlation between PGhr and age (C, controls).
Our study confirmed a correlation between total PGhr and GH levels in patients on dialysis (Table 4) that was previously reported in patients with less severe degrees of renal failure [5]. In contrast, we did not detect a significant correlation between PGhr and insulin. The physiological relationship between ghrelin and insulin has recently been a subject of controversy since ghrelin has been claimed to both stimulate [19] and inhibit [20] insulin secretion, whereas insulin appears to inhibit ghrelin secretion [13,16]. However, this inhibition of ghrelin secretion has been claimed to occur only at supraphysiological concentrations of insulin [15], and was not observed in another study [14].

We failed to detect a correlation between PGhr and residual renal function (Table 4). Our patients presented a markedly narrower spectrum of mean renal clearance than the patients in Yoshimoto et al. [5], which may have obscured any potential correlations between these parameters. In addition, the RIA method which may have obscured any potential correlations between these parameters. In addition, the RIA method [5] may have signalled retained ghrelin fragments which were not identified by the commercial RIA kit that we used.

We observed some minor differences in the correlations with PGhr between patients in the HD and PD groups. For instance, the correlation between PGhr and GH was stronger in PD patients, while the correlation between PGhr and BMI was statistically significant only in patients on HD (Table 4). The reason
for these differences is unclear and our study probably lacked the statistical power to analyse this question in depth.

The association between malnutrition, inflammation and atherosclerosis (MIA syndrome) in patients with renal failure has been a subject of marked interest in the last few years [21]. The pathogenesis of this syndrome is undoubtedly complex, but the possibility that inflammation may alter the physiologic mechanisms of appetite and weight regulation has received specific attention. For instance, inflammation may be associated with increased plasma leptin levels, causing a potential negative effect on appetite in chronic renal failure [6]. While investigating potential correlations between inflammation and PGhr levels, we could not find a crude or adjusted correlation between PGhr and the selected inflammatory markers (Table 4). These findings argue against a role for ghrelin as a mediator of the anorexia and malnutrition that frequently accompanies inflammatory states in chronic renal failure.

In our dialysis patients, we found a consistent inverse correlation between PGhr and common nutritional markers. This correlation was visible only after adjusting PGhr levels for age (Table 5). The significance of this finding is not totally clear, but suggests that ghrelin secretion increased in response to poor dietary intake, as indicated by the inverse correlation between ghrelin and protein intake (Table 5). If true, this mechanism would further argue against a disorder of ghrelin secretion as a cause of malnutrition in dialysis patients. However, resistance to the central effects of circulating ghrelin in these patients cannot be excluded from our data.

In conclusion, PGhr levels were moderately but significantly increased in patients undergoing HD and PD compared with normal, age-matched controls. These elevations were similar in HD and PD patients. Age was a strong determinant of PGhr levels, both in uraemic patients and in healthy controls. Dialysis adequacy, residual renal function and inflammation did not significantly influence ghrelin levels in these patients. The negative correlation between PGhr and nutritional markers suggests that ghrelin secretion is increased in response to low dietary intake in dialysis patients.

Acknowledgements. This work was supported by grants PGIDT00P01000PR (Xunta de Galicia, Spain) and PI021479 and GO3/028 (FIS of Instituto de Salud Carlos III, Spain).

Conflict of interest statement. The authors hereby declare not to have any conflict of interest which could raise the question of bias in the work reported, or in the conclusions, implications or opinions stated.

References

13. McCowen KC, Maykel JA, Bistrian BR, Ling PR. Circulating ghrelin concentrations are lowered by intravenous glucose or hyperinsulinemic euglycemic conditions in rodents. J Endocrinol 2002; 175: R7–R11