The relationship of plasma ghrelin level to energy regulation, feeding and left ventricular function in non-diabetic haemodialysis patients

Chia-Chu Chang¹,⁵, Ching-Hui Hung², Chaun-Shu Yen⁴, Kai-Lin Hwang⁶ and Ching-Yuang Lin³,⁵

¹Division of Nephrology and ²Division of Cardiology, Department of Internal Medicine, ³Department of Pediatrics, Children’s Hospital and ⁴Department of Medical Nutrition Therapy & Food Service, Changhua Christian Hospital, Changhua, ⁵Institute of Medical Research, Chang Jung Christian University, Taiwan and ⁶Department of Public Health, Chung Shan Medical University, Taichung, Taiwan

Abstract

Objective. Inadequate plasma ghrelin levels determine the suppression of appetite, nutritional state and morbidity. We investigated the correlation between plasma ghrelin levels and appetite, nutritional status and cardiovascular morbidity in maintenance haemodialysis (HD) patients.

Methods. We measured plasma ghrelin levels at 2 h intervals during a 24 h period in 22 non-diabetic HD patients, who were grouped into normal intake or disturbed intake according to subjective global assessments, and in six healthy controls.

Results. A significant positive correlation existed between the 24 h plasma ghrelin profile and most time-specific plasma ghrelin levels in non-diabetic HD patients. Ghrelin levels in the abnormal intake group were higher than those in the normal intake group. A significant positive correlation existed between ghrelin and left ventricle functions, including left ventricle mass index (r = 0.75, P = 0.008), left ventricle mass (r = 0.57, P = 0.03) and interventricular septum thickness (r = 0.70, P = 0.009). An inverse correlation existed between plasma ghrelin and right ventricular dimension (r = -0.45, P = 0.035). Body mass index (r = -0.64, P = 0.033) and body fat content (r = -0.619, P = 0.002) had significant negative correlations with plasma ghrelin.

Conclusions. Anorexia was found in patients with higher plasma ghrelin levels. Plasma ghrelin levels in non-diabetic HD patients showed a significant correlation with left ventricular function.

Keywords: appetite; end-stage renal disease; ghrelin; left ventricular function

Introduction

Anorexia in end-stage renal disease (ESRD) has many causes, which may include uraemia per se, as well as various co-morbidities and psychosocial factors. Malnutrition and loss of appetite are common symptoms in ESRD, and they are important predictors of prognosis. There are many peripheral signals, both short-term and long-term, that can greatly affect feeding behaviour. Short-term signals, such as cholecystokinin (CCK) and gastrointestinal stretch receptors, are involved in promoting a sensation of satiety that leads to meal termination. Long-term signals, insulin and leptin, are produced in proportion to recent energy intake and body adiposity [1].

Ghrelin, a novel 28 amino acid peptide of 3300 Da, and isolated mostly from the stomach, is an endogenous ligand for the growth hormone secretagogue receptor (GHSR) and potently stimulates growth hormone release [2]. It is also involved in energy homeostasis [3] and has orexigenic properties [4]. When administered either centrally or peripherally to rodents, ghrelin stimulates gastric motility and acid secretion. In rats, ghrelin reverses the anorectic effect of leptin, suggesting that there may be interplay between these two systems [5]. Recent data provide evidence that the rat arcuate nucleus integrates peripheral signals provided by leptin, insulin and ghrelin or ghrelin mimics [growth hormone-releasing protein-6 (GHRP-6)] to regulate food intake and body composition [6]. A marked pre-prandial increase in plasma ghrelin in humans has been reported, suggesting a physiological role in meal initiation [7]. In contrast, the permanent absence of food in the stomach, as in gastric bypass for weight control, ultimately causes more suppression of ghrelin than diet-induced weight loss [8]. After both ghrelin and GHSR were detected in the aorta and myocardium [9], the evidence supported the theory that
ghrelin had a role in myocardial growth and cardiac function.

On the one hand, it is well known that poor appetite and negative energy homeostasis are common in ESRD patients; on the other, that cardiovascular disease is the leading cause of mortality in these patients. We hypothesize that inadequate endogenous ghrelin levels disturb intake desire, causing cardiovascular complications in ESRD patients. Meanwhile, the diurnal pattern of plasma ghrelin levels has been defined in studies of healthy volunteers (7), but not in dialysis patients. In an attempt to test our hypothesis, we first serially measured 24 h plasma ghrelin levels in haemodialysis (HD) patients with or without poor appetite, and in healthy controls. Secondly, we studied the correlation between plasma ghrelin levels and left ventricular function in HD patients. We also investigated the correlation between plasma ghrelin and fat deposition that is representative of energy expenditure.

Patients and methods

Patients

We enrolled a cohort of 22 non-diabetic HD patients with anuria (mean age, 43.2±2.3 years; range, 62–35) comprised of 12 males (mean age, 39.4±0.9 years; range 42–37) and 10 females (mean age, 46.3±3.7 years; range 62–35); six age-matched volunteers were also recruited as controls. The patients were evaluated by government-licensed dietitians, and were divided, using subjective global assessments (SGAs), into a disturbed appetite group, those who sensed anorexia on more than one occasion per day, and a normorexic group, those who did not feel anorexia. Exclusion criteria included recent hospitalization (within 1 month) for acute or chronic disorders, age over 65 or under 18 years, weight change of >1 kg within 1 month or unwillingness to participate in the study. The Human Subjects Review Committee at the Chunghua Christian Hospital (CCH), Taiwan, approved all procedures and protocols, and informed written consent was obtained from all subjects before enrolment.

Study protocol

The investigation began in the CCH nephrology centre and lasted 24 h. After an overnight fast, patients came to our unit in the morning, and an intravenous catheter was placed in a forearm vein to draw blood at 2 h intervals, from 8 a.m. to 6 a.m. the next morning, for the measurement of plasma ghrelin levels. After plasma samples were taken, they were stored at −70°C for analysis. Plasma immunoreactive ghrelin was measured in duplicate with a radioimmunoassay (Phoenix Pharmaceuticals Inc., Belmont, CA) using a 125I-labelled bioactive ghrelin tracer and a rabbit polyclonal antibody against full-length, octanoylated human ghrelin. The assay can recognize the acylated and desacyl forms of the hormone. The lower and upper limits of detection were 100 and 1000 pg/ml. The coefficient of variation was <5% within assays and <14% between assays. Meals were provided at 8 a.m., 12 p.m. and 6 p.m. The energy density of each meal was 1.2 kcal/g, and daily total caloric supplement was 35 kcal/kg of ideal body weight, with a macronutrient distribution of 50% carbohydrate, 30% fat and 20% protein.

The left ventricular mass index (LVMI) of these subjects (left ventricular mass/body surface area, g/m²) was calculated by a cardiologist using a Hewlett Packard Sonos 2000® (Hewlett-Packard Inc., Andover, MA) Phased-Array Imaging System with the built-in software of the ultrasonographic unit. The wall motion score index (WMSI), a numeric scoring system adopted based on the contractility of the individual segment, was also calculated by dividing the sum of wall motion scores by the number of visualized segments to estimate the extent of myocardial ischaemia or infarction. Higher scores (from 1 to 5) indicated more severe wall motion abnormality. Left ventricle mass (LVM), interventricular septum thickness (IVSd), left ventricle posterior wall thickness (LVPW), right ventricular dimension (RVD) and ejection fraction (EF) were also obtained through standard procedures—parasternal long axis view, parasternal short axis view, apical four-chamber view and apical five-chamber view. Blood pressure was measured twice during the day with a random zero sphygmomanometer after a 5 min rest in the echocardiography unit. Pulse pressure was calculated as systolic blood pressure minus diastolic blood pressure.

We measured total protein, albumin (using the bromocresol green method), pre-albumin, lymphocyte count, cholesterol, triglycerides, ura, creatinine, ferritin, transferrin and haemoglobin by routine procedures with an automatic analyser. Fasting gastrin levels were measured with a commercial radioimmunoassay kit (MP Biomedicals, Inc., Orangeburg, NY) at the Department of Clinical Chemistry in CCH. The measure of the efficiency of the dialysis was the urea reduction rate (URR); the delivery dose of dialysis (Kt/Vurea) was calculated with a single-pool urea kinetic model. All patients were dialysed with FILTRYZER® (polymethylmethacrylate membrane, TORAY, Tokyo, Japan) and blood flow from 280 to 300 ml/min. For an indirect indicator of protein intake, normalized protein catabolic rate (PCRn) was calculated using dialysis urea removal and serum urea levels. The dietitian performed SGAs before meals. Body fat mass and fat free mass were directly measured via HITACHI® 600 Auto-analyser bioimpedance (Upwards Biosystems Ltd, Tanita, Osaka, Japan) after HD and before meals. Body mass index (BMI), fat mass index (FMI) and lean mass index (LMI) were then calculated by dividing body weight (kg), fat mass (kg) and lean mass (kg) by the square of height (m²), respectively.

Statistics

Ghrelin levels are expressed as mean and standard error (SE). The area under the curve (AUC) for the 24 h ghrelin profile was calculated with the trapezoidal rule. Pearson’s correlation coefficient was used to evaluate linear associations between continuous variables, 24 h AUC and ghrelin level at certain time points. A two-sample Student t-test was used to compare the differences between the 24 h AUC and continuous variables between patients and control groups. A statistical package, SAS v8.02 (SAS Institute Inc., Cary, NC) was used for analysis; and P-values <0.05 were considered statistically significant.
Results

Comparison of average plasma ghrelin levels

Plasma ghrelin levels measured at 2 h intervals averaged 20% higher in the HD group than in the normal control group at most time-specific points (Figure 1). Between the two groups, the plasma ghrelin levels were significantly different ($P < 0.05$) at 9.30 a.m.; the levels were the same at midnight and early morning, which was the period of long-term fasting. Comparing the 24 h AUCs of plasma ghrelin, that of the control group was 10 000.6±119.6 pg/ml and that of the patient group was 11 801.6±214.2 pg/ml; however, the difference between these two groups was not significant. Prior to each meal, plasma ghrelin levels would rise by 40% of pre-prandial levels in our patients. The nadir of plasma ghrelin appeared 1.5–2 h after each meal. The average of AUC of ghrelin levels of the HD group was 12 701±614.7 pg/ml in males and 11 052±246.8 pg/ml in females, an insignificant difference.

Correlation between 24 h AUC values and time-specific plasma ghrelin levels

There was a significant positive correlation between the AUC of 24 h ghrelin profiles and most time-specific ghrelin levels, except at 4 p.m. (Table 1). Especially during the long overnight fast and after breakfast (from 4 a.m. to 10 a.m.), the correlation coefficient reached 0.9. The reciprocal relationship was not, however, always present for all items that were measured at time-specific points or the 24 h AUC of ghrelin studied.

Plasma ghrelin in single haemodialysis clearance

The average plasma ghrelin clearance of a single HD was ~53%, with a URR of 72% (Table 2). Figure 2 shows serial plasma ghrelin levels during HD. More effective ghrelin clearance with a sharper slope was noted in the first 2 h of HD, and plasma ghrelin levels rebounded slightly later.

Correlation of plasma ghrelin with appetite and gastrin

The mean AUC of plasma ghrelin levels in the anorectic group were higher than in the non-anorectic group (12 877±345.1 vs 10 905±895.6 pg/ml). There was a negative correlation between plasma ghrelin levels and appetite ($r = -0.39, P = 0.23$) as evaluated using Pearson’s correlation coefficient. Patients with more gastrointestinal complaints (such as anorexia or nausea) seemed to have higher plasma ghrelin levels ($r = -0.34, P = 0.28$). Mean fasting plasma gastrin levels in our HD patients were 104.91±38.34 pg/ml, and their correlation coefficient with 24 h AUC of ghrelin levels was 0.218, which was insignificant.

Correlation of plasma ghrelin with nutrition and energy homeostasis

Analysing the varying nutritional parameters of HD patients, we found that serum albumin ($r = 0.32, P = 0.33$) had a stronger positive correlation with the

Table 1. Correlation between integrated AUC of 24 h ghrelin values and plasma ghrelin levels measured at 2 h intervals over 24 h in non-diabetic HD patients

<table>
<thead>
<tr>
<th>Sample time</th>
<th>Correlation coefficient</th>
<th>$P$-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>06:00</td>
<td>0.87888</td>
<td>0.0004**</td>
</tr>
<tr>
<td>08:00</td>
<td>0.88688</td>
<td>0.0003**</td>
</tr>
<tr>
<td>09:30</td>
<td>0.8519</td>
<td>0.0009**</td>
</tr>
<tr>
<td>10:00</td>
<td>0.88714</td>
<td>0.0006**</td>
</tr>
<tr>
<td>12:00</td>
<td>0.76227</td>
<td>0.0064</td>
</tr>
<tr>
<td>14:00</td>
<td>0.72774</td>
<td>0.0111*</td>
</tr>
<tr>
<td>16:00</td>
<td>0.56577</td>
<td>0.0697</td>
</tr>
<tr>
<td>18:00</td>
<td>0.62868</td>
<td>0.0383*</td>
</tr>
<tr>
<td>20:00</td>
<td>0.83729</td>
<td>0.0013**</td>
</tr>
<tr>
<td>22:00</td>
<td>0.85626</td>
<td>0.0032**</td>
</tr>
<tr>
<td>24:00</td>
<td>0.88671</td>
<td>0.0006**</td>
</tr>
<tr>
<td>02:00</td>
<td>0.83799</td>
<td>0.0013**</td>
</tr>
<tr>
<td>04:00</td>
<td>0.91891</td>
<td>0.0002**</td>
</tr>
</tbody>
</table>

Pearson’s correlation coefficient was used to evaluate the linear association, and $P$-values <0.05 were considered to be of statistical significance.$^*$ $P < 0.05; **P < 0.01.$

Table 2. Plasma ghrelin and blood urea clearances and their ratios in a single HD

<table>
<thead>
<tr>
<th></th>
<th>Ghrelin (pg/ml)</th>
<th>Blood urea (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre-dialysis$^a$</td>
<td>622.3±102.7</td>
<td>71.7±2.3</td>
</tr>
<tr>
<td>Post-dialysis$^a$</td>
<td>242.4±4.0</td>
<td>19.5±0.9</td>
</tr>
<tr>
<td>Clearance ratio$^b$</td>
<td>53.2±12.5 (%)</td>
<td>72.4±0.6 (%)</td>
</tr>
</tbody>
</table>

$^a$Values are presented as the mean±SE. $^b$The clearance ratio was defined as: pre-HD blood levels minus post-HD blood levels divided by pre-HD blood levels.
AUC of plasma ghrelin levels than pre-albumin ($r = 0.13$, $P = 0.7$) and total protein ($r = 0.039$). In contrast, body fat content ($r = -0.619$, $P = 0.002$) and BMI ($r = -0.64$, $P = 0.033$) had significantly negative correlations with the AUC of 24 h ghrelin values. The PCrN ($r = -0.37$, $P = 0.26$) and waist–hip ratio ($r = -0.40$, $P = 0.22$) both showed negative correlations with the AUC of ghrelin values.

Correlation of plasma ghrelin levels with left ventricular mass and function

As shown in Table 3, there was a significantly positive correlation between the AUC of plasma ghrelin levels and LVMi ($P < 0.01$), LVM ($P < 0.05$) and IVSd ($P < 0.01$). Otherwise, there was a significantly negative correlation between plasma AUC ghrelin levels and RVD ($P < 0.05$). Our study did not find a significant correlation between plasma ghrelin and cardiac EF ($P > 0.05$). We present a reciprocal correlation between LV functions and some time-specific plasma ghrelin levels in the 24 h series, between 6 a.m. and 10 a.m. (Table 4); there was an even more significant relationship between LV functions and plasma ghrelin levels at 8 a.m. or 9.30 a.m. Otherwise, the ESRD patients had higher AUC of plasma ghrelin levels, and their pulse pressure tended to be higher ($r = 0.44938$, $P = 0.1655$). However, there was no significant correlation between their systolic or diastolic blood pressures, lipid profile and AUC of plasma ghrelin levels (data not shown).

Likewise, there was also little correlation between plasma ghrelin levels, intact parathyroid hormone (iPTH), age, sex and erythropoeitin index (data not shown).

Table 4. Correlation of plasma ghrelin levels at specific points of time and left ventricular functions

<table>
<thead>
<tr>
<th>Time of Day</th>
<th>6 a.m.</th>
<th>8 a.m.</th>
<th>9.30 a.m.</th>
<th>10 a.m.</th>
<th>24 h AUC</th>
</tr>
</thead>
<tbody>
<tr>
<td>LVM</td>
<td>0.405</td>
<td>0.456*</td>
<td>0.610**</td>
<td>0.610**</td>
<td>0.570*</td>
</tr>
<tr>
<td>LVMi</td>
<td>0.573*</td>
<td>0.621***</td>
<td>0.741**</td>
<td>0.775**</td>
<td>0.750**</td>
</tr>
<tr>
<td>IVSd</td>
<td>0.612**</td>
<td>0.639**</td>
<td>0.745**</td>
<td>0.701**</td>
<td>0.703**</td>
</tr>
<tr>
<td>LVPW</td>
<td>0.044</td>
<td>0.440</td>
<td>-0.282</td>
<td>-0.433*</td>
<td>-0.543*</td>
</tr>
<tr>
<td>RVD</td>
<td>-0.451</td>
<td>0.035*</td>
<td>0.069</td>
<td>0.840</td>
<td></td>
</tr>
<tr>
<td>EF</td>
<td>-0.163</td>
<td>0.633</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Discussion

In our study, we first determined the profile of plasma ghrelin levels over 24 h, including an intra-dialysis period, in non-diabetic HD patients. We found a unique diurnal change: there was neither an obvious plasma ghrelin rise before each meal time nor a rapid fall after eating, as shown in previous reports [7]. We also found that the average level of plasma ghrelin in HD patients was one-fifth higher than in the healthy control group at most sampling times during the day, except between 10 a.m. and 2 a.m. These findings suggest that the kidney is an important site for clearance, degradation [10] or even production [11] of ghrelin; ghrelin mRNA expression did not change in the stomach of ESRD patients [11]. Some authors have reported that plasma ghrelin showed a significant correlation with serum creatinine levels, and it increased 2.8 times in patients with ESRD compared with patients with normal renal function [10]. The difference between plasma ghrelin levels in ESRD patients and healthy persons was greater in other studies than in ours [10], which may be attributable to different basal levels in different races. Yashimoto et al. [10] pointed out that $\sim 53.3\% \pm 8.3\%$ of ghrelin in...
plasma was removed from the blood with a single course of HD, and we found the same results (53.2 ± 12.5%). In contrast, leptin, one important, potent endogenous anorexic peptide of 16 kDa, would not be cleared by ordinary synthetic dialysis membranes or peritoneal dialysis [12].

In our study, the AUC of 24 h plasma ghrelin levels was larger, but not significantly, in the disturbed appetite group. The increased levels of ghrelin with an insufﬁcient orexigenic response would suggest that the ESRD patients have reduced basal hunger caused by constantly high plasma ghrelin levels or some resistance to its effect—either at the receptor or post-receptor level. Hyperleptinaemia, a common issue in patients undergoing chronic dialysis, also might counter-regulate the effect of ghrelin. In the genetic deletion model, ghrl−/− (ghrelin knock-out) mice exhibited normal spontaneous food intake patterns and normal growth rates [13]. The results indicated that endogenous ghrelin may not be a critical orexigenic factor. Besides ghrelin, numerous gastrointestinal hormones have been implicated in the regulation of food intake. G cells located in the lateral walls of the gastric antrum produce gastrin, which can stimulate gastric emptying and insulin secretion, controlled by the vagal nerve. In vitro, gastrin inhibits ghrelin release [14]. However, given in high doses, this hormone does not have any effect on ghrelin secretion in vivo [15].

In this study, we demonstrated that HD patients had normal fasting gastrin levels and there was no signiﬁcant correlation between ghrelin and gastrin. CCK, another gastrointestinal hormone that regulates food intake, has a satiating effect. One study showed that a higher plasma CCK was responsible for anorexia in the elderly [16]; we, however, did not check CCK levels in this study.

Cardiac disease is still the leading cause of death among patients on long-term dialysis, accounting for 44% of overall mortality among them. The prevalence of LVH in ESRD patients has been found to be as high as 75%. LVMI is the most reliable index for LVH, and our study also showed that plasma ghrelin levels (AUC) had a signiﬁcant positive correlation with LVMI and its individual components, LVM and IVSd. Plasma ghrelin failed to correlate with LVPW, another individual component of LVMI. This suggests that ghrelin’s effect on the myocardium was eccentric, via uncertain mechanisms. Recent data showed that ghrelin inhibited apoptosis in cardiomyocytes and endothelial cells through activation of extracellular signal-regulated kinase (ERK) 1/2 and Akt serine kinase [17]. In Table 4, we show that plasma ghrelin is inversely related to RVD, which expresses the right ventricular pressure or volume load. This implies that plasma ghrelin has some effects on pulmonary vasculature. One study proved that ghrelin possessed direct vasorelaxant properties by reversing endothelin-1-induced vasoconstriction [18]. In our study, both EF and WMSI, which represents the extent of regional wall motion abnormality due to myocardial ischaemia, failed to correlate with plasma ghrelin levels.

Long-term longitudinal studies may be needed to conﬁrm our results. As in ESRD patients, plasma ghrelin levels in cachetic patients with congestive heart failure are higher [19]. Collectively, these data demonstrate that an increase in plasma ghrelin might mediate compensatory mechanisms during catabolic–anabolic imbalance in HD patients with myocardial disease.

Among our patients, the AUC of 24 h ghrelin levels had a signiﬁcant negative correlation with BMI and body fat percentages. These ﬁndings are consistent with those of other studies in healthy groups [9]. Ghrelin was known to be involved, independent of food intake, in regulating energy balance via modulating preferentially the metabolic substrate (i.e., fat vs also failed to correlate with carbohydrate) used for the maintenance of energy balance [13]. The reduction of fat utilization after exogenous ghrelin administration was conﬁrmed in one study [20]. Moreover, we found that the AUC of plasma ghrelin levels had more correlation with long-term nutritional indices, albumin and ferritin, than with short-term nutritional parameters such as pre-albumin. To summarize, we believe that ghrelin is a hormone that signals the need to conserve energy to prevent cachexia or starvation.

The AUC of 24 h ghrelin values in HD patients correlated signiﬁcantly with ghrelin levels between 4 a.m. and 10 a.m., as reported in healthy volunteers [8]. This implies, as shown by Cummings et al. [7], that single measurements during that interval can be performed for future studies in ESRD patients, instead of 24 h blood sampling.

In conclusion, anorexia, instead of hyperphagia, was found in our patients with higher AUC of plasma ghrelin levels. These observations suggest that there is resistance to ghrelin action in ESRD patients, either peripheral or central, or both. Due to its signiﬁcant correlation with the composition of body fat, ghrelin could modulate the metabolic substrate and reduce fat utilization to maintain energy balance. There was a signiﬁcant correlation between the AUC of plasma ghrelin levels and most time-speciﬁc ghrelin levels. Plasma ghrelin levels are signiﬁcantly correlated with LVMI and RVD in ESRD patients. These links could be contributing to the high cardiovascular mortality in these patients. We suggest that the role of ghrelin elevation in ESRD patients with anorexia may be only a compensatory pathway rather than a causative factor.

Acknowledgements. The authors wish to thank certiﬁed nurse specialist Ms Tsai-Hung Chang for collecting samples. This study was supported by a grant (no. 9216) from the Changhua Christian Research Foundation.

Conflict of interest statement. None declared.

References

Role of ghrelin in left ventricular function in HD patients

6. Hewson AK, Tung LYC, Connell DW et al. The rat arcuate nucleus integrates peripheral signals provided by leptin, insulin, and a ghrelin mimetic. *Diabetes* 2002; 51: 3412–3419
16. Sturm K, MacIntosh CG, Parker BA et al. Appetite, food intake, and plasma concentration of cholecystokinin, ghrelin and other gastrointestinal hormone in undernourished older women and well-nourished young and older women. *J Clin Endocrinol Metab* 2003; 88: 3747–3755