Ghrelin and other appetite-regulating hormones in paediatric patients with chronic renal failure during dialysis and following kidney transplantation

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Abstract

Background. Patients with renal insufficiency often suffer from cachexia and growth retardation due to low appetite and increased resting metabolic rate. The neuroendocrine hormone ghrelin, a growth hormone secretagogue, enhances food intake, but its role in the development of a cachectic state in renal insufficiency is unclear.

Objective. The aim of our study was to investigate the plasma concentration of total ghrelin and other hormones involved in appetite regulation in children with preterminal chronic renal failure (CRF, n = 24), children undergoing dialysis (n = 19), children after renal transplantation (RTx, n = 59) and healthy controls (n = 10).

Results. Total ghrelin was significantly elevated in CRF patients (1370 ± 182 pg/ml; mean ± SEM) when compared to control subjects (682 ± 106 pg/ml; P = 0.016) or patients following RTx (859 ± 51 pg/ml; P = 0.002). Furthermore, a negative correlation between glomerular filtration rate and total ghrelin was observed in CRF and transplant recipients (r = 0.36, P = 0.0006). BMI SDS (standard deviation score) is lower in CRF patients compared to the other groups (P < 0.0001). Leptin, adiponectin, blood glucose, insulin, IGF-I, IGFBP-3 and growth hormone concentrations did not differ among groups.

Conclusions. We observed elevated ghrelin levels in uraemic patients despite poor appetite, but the underlying reasons remain unclear. Normal ghrelin levels can be re-achieved following RTx.

Keywords: appetite dysregulation; CRF; children; ghrelin; RTx

Introduction

Ghrelin, a recently discovered 28-amino-acid polypeptide with a molecular weight of 3kD, mainly produced in the stomach, is one of the most potent orexigenic hormones [1,2]. Its receptor, the growth hormone secretagogue receptor 1a (GHS-R1a), is mainly expressed in the pituitary and hypothalamus [3]. Binding of the acylated active form of ghrelin to this receptor mediates appetite regulation. In the circulation, the non-acylated form of ghrelin predominates with ~90% of the total ghrelin [4].

One major effect of ghrelin is the increase of food intake with a subsequent increase of body weight, fat accumulation and increase in carbohydrate oxidation [5,6]. This is exerted by activating neuropeptide Y (NPY) and agouti-related peptide (AgRP) producing neurons localized in the arcuate nucleus. Furthermore, ghrelin is involved in the regulation of growth hormone (GH) secretion using a specific, GH-releasing hormone (GHRH) independent pathway [7]. Peripheral ghrelin concentrations are elevated during starvation and decrease within 30–60 min after a meal [8]. Ghrelin plasma levels show a negative correlation with body mass index (BMI) [9,10] and age, the decrease beginning in early childhood [9].

Paediatric patients with preterminal chronic renal failure (CRF) often suffer from insufficient weight gain and growth retardation due to low appetite and low calorie intake. These children have an increased resting metabolic rate and show signs of chronic inflammation, particularly within the first 2 years of life. Often nasogastric tube feeding is required to provide sufficient nutritional intake [11]. This uraemic malnutrition is associated with high morbidity, poor quality of life and increased mortality rates. After renal transplantation (RTx) with normalization of renal function, an improvement in the appetite dysregulation is seen followed by catch-up growth as well as improvement in quality of life and outcome [12].

Plasma levels of total ghrelin have been shown to be increased in uraemic adults and children [4,13,14]. However,
little is known about the regulation of ghrelin in children with CRF and during dialysis treatment since all studies included only few paediatric patients. Data on renal transplant recipients do not exist to date. Total and active ghrelin are known to be cleared through the kidney. In haemodialysis patients, ghrelin is cleared sufficiently with haemodialysis [4,14].

Our aim was to analyse the changes in total ghrelin in paediatric patients with acute and chronic renal diseases (CRF, receiving dialysis treatment and after RTx) to obtain more information about the causes of malnutrition in uraemic patients.

Patients and methods

Patients and controls

A total of 102 patients with a mean age of 10.3 years (0–18 years) were included in our study: 24 patients with CRF, 19 children undergoing dialysis treatment (haemodialysis \( n = 8 \), peritoneal dialysis \( n = 11 \)) and 59 children following RTx. Ten healthy children with a mean age of 7.9 years (1–15 years) served as controls. Patients were diagnosed with obstructive uropathy \( (n = 17) \), hyperoxaluria type I \( (n = 2) \), nephrotic syndrome \( (n = 12) \), nephronophthisis \( (n = 6) \), renal hypo/dysplasia \( (n = 26) \), haemolytic uraemic syndrome \( (n = 9) \), autosomal recessive polycystic kidney disease \( (n = 3) \), Alport syndrome \( (n = 1) \), autoimmune kidney disease \( (n = 5) \), renal insufficiency due to hereditary syndromes \( (n = 10) \), reflux nephropathy \( (n = 2) \), neurogenic uropathy \( (n = 3) \), cystinosis \( (n = 1) \), Schoenlein-Henoch disease \( (n = 3) \) and other renal disorders \( (n = 2) \).

Management of blood samples

Blood samples were collected in the morning at least 4 h after the last food intake into EDTA-coated tubes (Sarstedt, Germany). Tubes were instantly cooled on ice and centrifuged at 3000 rpm for 10 min at 4°C within 30 min. Plasma was stored at −70°C. In patients receiving haemodialysis, blood samples were collected in the morning before haemodialysis and in patients with peritoneal dialysis after the first daytime dialysate exchange in the morning.

Laboratory methods

Measurement of ghrelin plasma levels. Total ghrelin concentrations (pg/ml) in blood samples were measured with RIA using polyclonal rabbit antibodies raised against human ghrelin (100% specificity for Octanoyl-Ser\(^3\)-Ghrelin, Des-Octanoyl-Ser\(^{-3}\)-Ghrelin and Gin\(^{28}\)-Ghrelin, intra-assay variability 4.0%, inter-assay variability 7.5%) (Phoenix Pharmaceuticals, Belmont, CA, USA).

Measurement of GH, adiponectin, leptin, IGF-I, IGFBP-3 and insulin levels. As an anabolic hormone, GH plays a major role in renal insufficiency and is used to promote growth and normalize body composition in children with CRF [15]. Furthermore, ghrelin contributes to the regulation of GH concentrations [7]. We wanted to test whether a relationship exists between simultaneously measured GH and other metabolically important hormones and ghrelin concentrations in children with CRF.

Growth hormone, adiponectin, leptin, IGF-I, IGFBP-3 and insulin levels were measured using commercially available assays and ELISA according to the manufacturers’ instructions: GH immunoassay (Nichols Institute Diagnostika, Bad Vilbel, Germany); Adiponectin ELISA (Linco Research, St. Charles, MO, USA); Leptin ELISA (Linco Research, St. Charles, MO, USA); IGF-I immunoassay (Nichols Institute Diagnostika, Bad Vilbel, Germany); Insulin immunoassay (DPC Biemann, Bad Nauheim, Germany); IGFBP-3 immunoassay (Mediagnost, Reutlingen, Germany).

Calculation of glomerular filtration rate (GFR). Creatinine is expressed as mg/dl, and glomerular filtration rate (GFR) is calculated according to the Schwartz formula [16].

Statistical analysis

Comparisons of continuous variables are made with unpaired Wilcoxon tests, one-way ANOVA and Bonferroni’s multiple comparison test and are given as mean ± SEM. The level of statistical significance was predefined as \( \alpha = 0.05 \), and resulting \( P \)-values are given descriptively. Correlations were performed by linear regression analysis using Pearson’s correlation coefficient. Our data were tested for normal distribution using the D’Agostino-Pearson normality test. The statistical analysis was performed using GraphPad Prism\textsuperscript{©} (version 5.01 for windows, GraphPad Software, San Diego, CA, USA).

Ethics approval

The study was approved by the ethics committee of the University Duisburg-Essen. Patients and controls were entered into the study after having obtained written informed consent from the parents and if appropriate also from the patients.

Results

Patients and controls showed no significant group differences regarding gender and age (Table 1).

Patients with CRF and patients undergoing haemodialysis (HD) and peritoneal dialysis (CAPD) treatment showed a significantly lower BMI SDS (standard deviation score) when compared to patients following RTx and to controls \( (P < 0.0001; \text{result of a five-group ANOVA; post hoc statistical analysis; Figure 1}) \). Serum albumin as another objective marker of malnutrition was not different between groups (data not shown).

Total plasma ghrelin concentrations were significantly elevated in CRF patients \( (1023 ± 116 \text{ pg/ml}) \) when compared to healthy controls \( (538 ± 91 \text{ pg/ml}; P = 0.016) \) and children following RTx \( (689 ± 46 \text{ pg/ml}; P = 0.002; \text{post hoc statistical analysis; Figure 2}) \).
Table 1. Characteristics of patients and healthy children as controls

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>Sex ratio (male:female)</th>
<th>Age (years) (mean ± SEM)</th>
<th>BMI SDS (mean ± SEM)</th>
<th>GFR&lt;sub&gt;Schwartz&lt;/sub&gt; (ml/min/1.73 m²) (mean ± SEM; range)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>10</td>
<td>7:3</td>
<td>7.9 ± 4.9</td>
<td>0.04 ± 1.8</td>
<td>98.4 ± 5.8 (82–104)</td>
</tr>
<tr>
<td>CRF</td>
<td>24</td>
<td>17:8</td>
<td>7.9 ± 5.8</td>
<td>−0.6 ± 0.9</td>
<td>15.7 ± 6.2 (7–28)</td>
</tr>
<tr>
<td>CAPD</td>
<td>11</td>
<td>6:5</td>
<td>6.9 ± 5.0</td>
<td>−0.7 ± 1.0</td>
<td>13.7 ± 5.0 (7–21)</td>
</tr>
<tr>
<td>HD</td>
<td>8</td>
<td>6:2</td>
<td>14.4 ± 1.4</td>
<td>−0.5 ± 1.1</td>
<td>11.8 ± 2.1 (9–18)</td>
</tr>
<tr>
<td>RTx</td>
<td>59</td>
<td>39:21</td>
<td>12.4 ± 1.4</td>
<td>0.3 ± 1.1</td>
<td>66.9 ± 21.3 (41–127)</td>
</tr>
</tbody>
</table>

Fig. 1. BMI SDS in patients with preterminal chronic renal failure (CRF), under dialysis treatment (CAPD, HD), after renal transplantation (RTx) and in healthy controls. BMI SDS is significantly reduced in patients with CRF and under dialysis compared to patients after RTx and healthy controls (controls versus CRF <i>P</i> < 0.05; CRF versus RTx, HD versus RTx, CAPD versus RTx <i>P</i> < 0.01; Bonferroni’s multiple comparison test). All other comparisons were not significantly different. Boxes indicate the median and the 25th–75th percentile range.

Fig. 2. Plasma levels of total ghrelin (pg/ml) in patients with preterminal chronic renal failure (CRF), under dialysis treatment (CAPD, HD), after renal transplantation (RTx) and in healthy controls. Total ghrelin levels are significantly increased in patients with CRF and under dialysis compared to RTx patients and healthy controls. Boxes indicate the median and the 25th–75th percentile range.

There was a significant correlation between BMI SDS and plasma levels of total ghrelin in the group of patients after RTx (<i>r</i> = −0.31; <i>P</i> = 0.02 <i>n</i> = 54; Figure 3).

In the patient groups who did not receive dialysis treatment (CRF, renal transplant recipients and healthy controls), we could find a significant correlation between the GFR<sub>Schwartz</sub> and the plasma level of total ghrelin (<i>r</i> = −0.36; <i>P</i> = 0.006 <i>n</i> = 89; Figure 4).

In our studies, no significant difference was found among the groups for leptin, adiponectin, insulin-like growth factor I (IGF-I), insulin-like growth factor binding protein-3 (IGFBP-3) and insulin and growth hormone (data not shown). However, we could detect a trend towards elevated plasma levels of IGF-I and leptin in patients with CRF (leptin: CRF = 12.4 ± 3.9 ng/ml versus all others = 9.6 ± 1.1 ng/ml, <i>P</i> = 0.33; IGF-I: CRF = 3.09 ± 0.47 ng/ml versus all others = 2.37 ± 0.15 ng/ml, <i>P</i> = 0.05).

Discussion

We studied plasma levels of total ghrelin in paediatric patients with CRF undergoing dialysis and following RTx in comparison to healthy controls. Our data show that total ghrelin is significantly increased in CRF children who present with low appetite and malnutrition. This phenomenon has previously been observed in adult patients [4], but only small series with few paediatric patients...
exist [14]. Our cross-sectional data also reveal that following RTx, plasma levels of total ghrelin decline to ghrelin levels of healthy children with subsequent development of good appetite and weight gain. We are aware of the limitations of our study such as small sample size of paediatric control subjects and CRF patients. However, to our knowledge, this is the first study to provide data of ghrelin levels in children following RTx and by far the largest published population of uraemic paediatric patients.

In addition, we could detect a trend towards elevated plasma levels of IGF-I and leptin in patients with CRF. These results would be compatible with those of other groups, who reported on elevated leptin plasma levels in children with CRF [17,18]. Elevated plasma levels of leptin in uraemic patients might also explain reduced appetite in these patients. However, we did not see a significant correlation between leptin and GFR_Schwartz in our cohort. In order to make a qualitative assessment of appetite, each patient or parent received a nutritional protocol with an observation period of 1 week. Unfortunately, only few and in most cases incomplete protocols were returned making any statistical analysis impossible.

The underlying pathomechanism remains unclear so far. It is not the plasma level of the acylated, active form of ghrelin that is raised in uraemic patients, but instead the level of the non-acylated ghrelin [4]. Both ghrelin forms are detected with commercial ELISA assays as total ghrelin. In contrast to acylated (active) ghrelin which is known to increase food intake in humans and rats, no direct effect of non-acylated ghrelin to increase appetite has been shown so far. In contrast, high plasma levels of non-acylated ghrelin have been shown to inhibit appetite in mice [19]. Therefore, it is feasible to speculate that the high levels of non-acylated ghrelin in uraemic patients may contribute to the poor appetite in these patients.

Another explanation for the reduced appetite in the face of high ghrelin levels in uraemic patients might be a different expression of the ghrelin precursor protein obestatin. Obestatin is a direct ghrelin regulator and opposes ghrelin action by suppressing food intake [20]. However, this remains unanswered at this time since there is no existing data on the regulation of obestatin in CRF. One could speculate that poor appetite, low caloric intake and growth retardation in children with CRF may be regarded as a protective mechanism to survive renal insufficiency by reducing the accumulation of toxic metabolites. Further studies addressing these questions are in progress.

Conclusions

We found significantly elevated plasma levels of total ghrelin in patients with CRF when compared to healthy controls or patients following RTx. Despite these elevated ghrelin levels, the appetite in our CRF patients was reduced. This is in contrast to observations from patients with non-uraemic conditions. After RTx, appetite improves and plasma levels of total ghrelin decrease.

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Conflict of interest Statement. None declared.

References


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