Hormonal regulation of energy-protein homeostasis in hemodialysis patients: an anorexigenic profile that may predispose to adverse cardiovascular outcomes

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Suneja M, Murry DJ, Stokes JB, Lim VS. Hormonal regulation of energy-protein homeostasis in hemodialysis patients: an anorexigenic profile that may predispose to adverse cardiovascular outcomes. Am J Physiol Endocrinol Metab 300: E55–E64, 2011. First published October 19, 2010; doi:10.1152/ajpendo.00438.2010.—To assess whether endocrine dysfunction may cause derangement in energy homeostasis in patients undergoing hemodialysis (HD), we profiled hormones, during a 3-day period, from the adipose tissue and the gut and the nervous system around the circadian clock in 10 otherwise healthy HD patients and 8 normal controls. The protocol included a 40-h fast. We also measured energy-protein intake and output and assessed appetite and body composition. We found many hormonal abnormalities in HD patients: 1) leptin levels were elevated, due, in part, to increased production, and nocturnal surge in response to daytime feeding, exaggerated. 2) Peptide YY (PYY), an anorexigenic gut hormone, was markedly elevated and displayed an augmented response to feeding. 3) Acylated ghrelin, an orexigenic gut hormone, was lower and did not exhibit the premeal spike as observed in the controls. 4) neuropeptide Y (NPY), a potent orexigenic peptide, was markedly elevated and did not display any circadian variation. 5) Norepinephrine, marginally elevated, did not exhibit the normal nocturnal dip. By contrast, α-melanocyte-stimulating hormone and glucagon-like peptide-1 were not different between the two groups. Despite these hormonal abnormalities, HD patients maintained a good appetite and had normal body lean and fat mass, and there was no evidence of increased energy expenditure or protein catabolism. We explain the hormonal abnormalities as well as the absence of anorexia on suppression of parasympathetic activity (vagus nerve dysfunction), a phenomenon well documented in dialysis patients. Unexpectedly, we noted that the combination of high leptin, PYY, and NPY with suppressed ghrelin may increase arterial blood pressure, impair vaso-dilatation, and induce cardiac hypertrophy, and thus could predispose to adverse cardiovascular events that are the major causes of morbidity and mortality in the HD population. This is the first report attempting to link hormonal abnormalities associated with energy homeostasis to adverse cardiovascular outcome in the HD patients.

For at least the past two decades, investigators have reported that protein energy malnutrition is prevalent in patients with chronic kidney diseases (CKD) and that the poor nutritional state contributes to the high morbidity and mortality (33, 34, 53, 54). Recent studies (28, 48, 61) further suggest that cachexia, the loss of lean body mass that is refractory to treatment with dietary supplementation, is also common in the CKD population. Anorexia is believed to be a major contributing factor to the malnutrition, (10–12, 25). The etiologies of anorexia and cachexia have been attributed to many factors including retention of uremic toxins, metabolic acidosis, and increased magnitude of inflammation and oxidative stress (6, 9, 26, 42, 64). After the discovery of leptin, its level was found to be elevated in CKD patients and many proposed that this increase may contribute to the loss of appetite (24, 36, 60).

However, whether high leptin is causally linked to anorexia remains unclear. Fueled by the successful cloning of leptin, a new roadmap of energy homeostasis emerged and a large number of peptides were found to interact with leptin. Marks et al. (45) reported an important role of the central melanocortin system in cachexia. Subsequently, Cheung et al. (14, 15) showed that 5/6 nephrectomy (Nx) in mice resulted in a reduction in food intake, loss of total body lean and fat mass, and an increased resting metabolic rate. These manifestations were blunted in db/db mice, i.e., mice with defective leptin receptors. Furthermore, mice lacking the melanocortin 4 receptor (MC-4R), a major target for α-MSH, also had a blunted metabolic response to Nx as did mice with intracerebroventricular injection of agouti-related peptide (AgRP), an MC-4R antagonist. These results suggest that high leptin may lead to increased melanocortin-4 activity and consequently cachexia in Nx mice.

These studies in the rodents inspired us to examine the hormonal status regulating energy homeostasis in hemodialysis (HD) patients. We profiled hormones originating from the adipose tissue, the gut, and the nervous system. Because many of these hormones have a diurnal pattern and meal-related changes, we measured these hormones around the circadian clock and included the perturbation of a 40-h fast, followed by 20 h of refeeding. Simultaneously, we measured caloric and protein intake and output, measured body composition, and assessed appetite and general health status. To avoid the compounding effects of concomitant illnesses and old age and to focus precisely on the effect of uremia, we strictly chose HD patients who were totally free of concomitant diseases and were relatively young.

METHODS

The protocol was approved by the Institutional Review Board at Carver College of Medicine, University of Iowa.

Study subjects. Ten HD patients and eight normal subjects were recruited. The HD patients had the following diagnoses: four patients had focal segmental glomerulosclerosis, one each had hypertensive nephrosclerosis, combined hypertension and obstructive uropathy, chronic interstitial nephritis, adult polycystic kidney disease, nail-patella syndrome, and lupus nephritis; the latter patient had no evidence of active disease at the time of this study. All patients were clinically stable and without any concurrent illnesses. HD was
performed three times weekly, 3–4 h each session, using CT190G dialyzers; \(kt/v\) (urea kinetics) ranged from 1.3 to 1.5. During the study week, because of time constraint of the study protocol, all dialysis was carried out in 3-h sessions. Hemodialysis was performed on Monday and Wednesday morning during the General Clinical Research Center (GCRC) stay. After discharge on Thursday, the HD patients returned on Friday morning for the third session. Patients with comorbid illnesses including cancer, heart failure, diabetes, or other systemic illnesses were excluded, and all patients had hemoglobin of \(\geq 11\) g/dl.

Subjects made one visit to the GCRC dietitian to create a menu for the study period that would mimic his/her routine intake. As illustrated in Fig. 1, subjects were placed on this diet for 3 days and were admitted to the GCRC on the afternoon of day 1, Monday, when a heparin lock was placed for blood draws. After the snack at 2000, food was withheld for 40 h until 1200 on day 3 (Wednesday). At that time, feeding was resumed and study continued until day 4, 0800 (Thursday). During the fast, water was allowed ad libitum. Subjects were encouraged to maintain their routine physical activities as much as permitted by the constraint of the protocol. During refeeding, the menu remained unchanged, but portion size was ad libitum. The amount of food ingested before fasting and during refeeding was recorded. Blood samples for hormone assays were taken at times shown in Fig. 1.

Nutritional assessment. The amount of lean and fat mass was measured by the whole-body dual energy absorptiometry scanner using the Hologic Delphi A Upgrade Model with software XP12.4 (Bedford, MA).

The subjective global assessment form was completed on every subject. It is a clinical tool used to assess nutritional status based on features of the history and physical examination. The former includes weight change, dietary change, and gastrointestinal symptoms, and the latter includes evidence of subcutaneous fat loss, muscle wasting, and edema or ascites. The scores are as follows: A, well nourished; B, moderately malnourished; and C, severely malnourished (19).

Appetite assessment. We assessed appetite with the visual analog scale (VAS) utilizing five questions (46): How hungry do you feel? How full does your stomach feel? How do you feel nauseous? How strong is your desire to eat? How much food do you think you can eat? Each question was rated on a scale of 1–10 and was measured three times a day during the study period before meal times.

Energy and protein balance. Calorie and protein intake were measured directly from the subjects’ food consumption at the GCRC. Resting energy expenditure was measured by \(O_2\) consumption (\(V_O_2\)) and \(CO_2\) production (\(V_{CO_2}\)) using a ventilated hood with the Vmax Spectra 22 machine from Sensor Medicis (Yorba Linda, CA). Resting energy expenditure was measured three times on each study subject, after an overnight fast, after a 36-h fast, and 30 min after completion of lunch during refeeding. Protein catabolism was measured in the control subjects by collecting 24-h urine for urea nitrogen (ureaN) excretion. The first collection started on day 1 at home and was completed the next morning at the GCRC; this collection represented the ureaN output at baseline. The second collection was from day 2 to day 3, representing ureaN output during fasting, and the third one, from day 3 to day 4, representing ureaN output during refeeding. For the HD patients, ureaN output was derived using urea kinetics (21); \(kt/v\) and normalized protein nitrogen appearance rate (nPNA) on day 1 (Monday), day 3 (Wednesday), and day 5 (Friday) of the study week. The Monday nPNA represented the baseline ureaN output from the end of the hemodialysis on the preceding Friday to that Monday noon. The Wednesday value represented the ureaN output during fasting, and the Friday value represented ureaN output during refeeding. In addition to \(kt/v\) and nPNA, we also made correction of the urea nitrogen output by calculating the changes in ureaN pool at the end of each dialysis session using the following equation (38):

\[
\Delta\text{ureaN pool} = (\text{BUN}_i - \text{BUN}_f) \times \text{Wti} \times 0.6 + (\text{Wt}_i - \text{Wt}_f) \times \text{BUN}_f
\]

\(\text{BUN}_i\) and \(\text{BUN}_f\) represented blood urea nitrogen (g/l) at the end of the current HD and the end of previous HD, and \(\text{Wt}_i\) and \(\text{Wt}_f\) represented body weight (kg) at the end of the current and the end of the previous session, respectively. If body urea nitrogen pool was reduced during the period of observation, that meant the urea kinetics had overestimated the protein catabolic rate and that amount was subtracted from the calculated nPNA. Vice versa, if the urea pool was increased, then that value was added to the calculated nPNA.

Hormone and other assays. We measured the following hormones:

1) leptin: RIA using the Linco kits (Linco, St. Charles, MO). The antiserum is highly specific for human leptin.
2) Peptide YY (PYY): RIA using Linco kits with antibody that recognizes both the 1–37 and 3–36 forms of human PYY.
3) Acylated ghrelin: RIA kits from Linco Research using antibody specific for the biologically active form of ghrelin with an octanoyl group on serine 3.
4) Glucagon-like peptide-1 (GLP-1): measured by enzyme-linked immunoassay using kits from ALPCO Diagnostic (American Laboratory Product, Salem, NH), which were highly specific for the immunologic measurement of active GLP-1 (7–36). 5) α-Melanocyte-stimulating hormone (α-MSH): measured by RIA with kits from ALPCO Diagnostic.
6) Neuropeptide Y (NPY): measured by RIA kits from ALPCO Diagnostic.
7) Norepinephrine was measured by a reverse-phase column which separates the individual catecholamines. Detection was accomplished by the use of a Coulochem II Dual Potentiostat Electro-chemical Detector (ESA, Chelmsford, MA).

CRP was measured by a sandwich enzyme-linked immunoassay using kits from ALPCO Diagnostics which consisted of polyclonal antibodies directed against CRP and a peroxidase-labeled CRP antibody. TNFα was measured using enzyme-linked immunoassay kits from R & D Systems (Minneapolis, MN), which contain Escherichia coli-derived recombinant human TNFα.
Blood draws. Blood was drawn into syringes, and predetermined amounts were emptied into several EDTA tubes containing specific and appropriate amounts of either aprotinin or DDP 4 inhibitor as indicated. Tubes of blood were then centrifuged at 4°C, and predetermined volumes of plasma were aliquoted into prelabeled 12 × 75 mm polypropylene tubes and kept frozen at −70°C until time of assay; all syringes and tubes were chilled before use. A total of 106 tubes of plasma samples were obtained from each study subject, and all were stored in clearly labeled tubes with participant’s initial, date, time, and peptide to be assayed.

Statistics and kinetics. Values are presented as means ± SE. For comparison of each time point values between the control and the HD groups, we used the Student’s t-test. For comparison of values within each group during the study period, we used the repeated-measures one-way ANOVA. To examine the relationship between plasma leptin and body fat, we used the standard linear regression analysis. The kinetic parameters for leptin and PYY were analyzed using a single compartmental approach. The disappearance rate was calculated from the peak during baseline to the end of fasting, whereas the appearance rate was derived from the area under the curve (AUC) from the end of fasting to the peak after refeeding. Maximal and minimal plasma concentrations (Cmax and Cmin), starting from the end of fasting to the peak post refeeding, were obtained by visual inspection of the concentration-time curves. To predict the factors that influenced Cmax, we performed a stepwise forward and reverse linear regression analysis using the statistical package of JMP 8 (SAS, Cary, NC).

RESULTS

Demography, body composition, inflammatory status, and baseline hormone levels. Table 1 shows that age, body mass index, and weight were comparable in both study groups; there was, however, an uneven sex distribution, more women in the control group. The two groups also had similar amounts of fat and lean body masses. The inflammatory markers including CRP and TNFα tended to be higher in the HD patients but did not reach a statistically significant threshold. Serum albumin was normal in both groups. Subjective global assessment, not listed in the table, placed all participants, controls as well as the control group. The two groups also had similar amounts of fat and lean body masses. The inflammatory markers including CRP and TNFα tended to be higher in the HD group. Molecular weights of the peptides were listed to show a lack of relationship between molecular weight and any increase in plasma levels in the HD patients.

Leptin profile and kinetics. Figure 2A depicts leptin rhythm throughout the study. There is a very distinct circadian rhythm with a nocturnal surge at ~0300 during feeding. Fasting totally abolished the nocturnal rise and led to a progressive decline of leptin to as low as 38 and 24% of the peak nocturnal levels, respectively, in the control and HD groups. During feeding, HD patients had higher plasma leptin levels and more robust nocturnal surges; in fasting, levels were not different between the groups.

Figure 2B illustrates a significant positive linear regression between plasma log leptin, values obtained on day 2, 0800, and percent body fat in both the control and the HD groups. Although the two lines showed that for each amount of body fat, HD patients had higher plasma leptin than the controls, but statistically, neither the log leptin-percent body fat regression lines nor the intercepts reached significant threshold.

Table 3 summarizes leptin kinetics showing that its elimination half-lives, 1/2α and 1/2β, were not different between the two groups. The AUC, derived from the end of fasting to the peak after refeeding, which we used as a surrogate for its secretory capacity, tended to be greater in the HD group, although the difference did not achieve a statistically significant threshold. Similarly, Cmax and Cmin, derived from visual inspection of the concentration-time curves, were higher in the HD group but again did not achieve a statistically significant level. By contrast, the AUC, Cmax and Cmin of male and female participants from both study groups combined showed significantly higher values in the females. While conventional statistics failed to reveal a significant difference in the secretory rate between the controls and the HD patients, with the use of multi-factorial regression analysis with the JMP 8 statistical package

Table 1. Demography, body composition, and inflammatory markers

<table>
<thead>
<tr>
<th>Sex</th>
<th>M</th>
<th>F</th>
<th>Age, yr</th>
<th>BMI</th>
<th>Weight, kg</th>
<th>LM, %</th>
<th>Body Fat, %</th>
<th>Trunk Fat, %</th>
<th>CRP, mg/l</th>
<th>TNFα, pg/ml</th>
<th>Albumin, g/dl</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>3</td>
<td>5</td>
<td>48 ± 6</td>
<td>28 ± 2</td>
<td>80 ± 5</td>
<td>66 ± 0.03</td>
<td>31 ± 3</td>
<td>31 ± 4</td>
<td>2.1 ± 0.9</td>
<td>1.7 ± 0.7</td>
<td>4.3 ± 0.6</td>
</tr>
<tr>
<td>HD</td>
<td>7</td>
<td>3</td>
<td>41 ± 5</td>
<td>28 ± 2</td>
<td>83 ± 6</td>
<td>69 ± 0.03</td>
<td>29 ± 3</td>
<td>29 ± 3</td>
<td>4.5 ± 1.8</td>
<td>9.1 ± 4.8</td>
<td>4.2 ± 0.06</td>
</tr>
</tbody>
</table>

Values are means ± SE. HD, hemodialysis patients; BMI, body mass index; LM, lean mass; CRP, C-reactive protein; NS, not significant. P values were derived from Student’s t-test.

Table 2. Baseline hormone values*

<table>
<thead>
<tr>
<th>Peptide</th>
<th>Molecular Mass, kDa</th>
<th>Control</th>
<th>HD</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leptin, ng/ml</td>
<td>16.00</td>
<td>11.2 ± 3.4</td>
<td>32.0 ± 8.9</td>
<td>0.05</td>
</tr>
<tr>
<td>PYY, pg/ml</td>
<td>4.31</td>
<td>98.3 ± 8.8</td>
<td>441 ± 35</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Acy-ghrelin, pg/ml</td>
<td>3.37</td>
<td>103.4 ± 22.5</td>
<td>85.6 ± 15.7</td>
<td>0.51</td>
</tr>
<tr>
<td>GLP-1, pmol/l</td>
<td>4.11</td>
<td>9.9 ± 4.3</td>
<td>6.7 ± 3.6</td>
<td>0.58</td>
</tr>
<tr>
<td>α-MSH, pmol/l</td>
<td>4.54</td>
<td>12.7 ± 1.6</td>
<td>12.9 ± 1.5</td>
<td>0.95</td>
</tr>
<tr>
<td>NPY, pmol/l</td>
<td>4.27</td>
<td>65.9 ± 6.5</td>
<td>137.1 ± 8.8</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Norepinephrine, pg/ml</td>
<td>0.17</td>
<td>445 ± 101</td>
<td>643 ± 225</td>
<td>0.47</td>
</tr>
</tbody>
</table>

*Values are derived from blood samples taken on day 2, 0800, after an overnight fast and are means ± SE. A lack of relationship between molecular mass and any increase in plasma level found in the HD patients is shown. PYY, polypeptide Y; GLP, glucagon-like peptide; α-MSH, α-melanocyte-stimulating hormone. P values were derived from Student’s t-test.
(SAS), stepwise forward and reverse regression analysis showed that three factors significantly predicted higher leptin C\text{max}: group, higher in the HD patients ($P = 0.005$); sex difference, higher in the female sex ($P = 0.05$); and percent trunk fat, higher in those with greater amount of trunk fat ($P = 0.002$).

Profile of the gut-derived hormones. The three gut hormones that we studied included PYY, acylated ghrelin, and GLP-1. Figure 3A shows that circulating PYY levels were fourfold higher in the HD patients at all time points. In both groups, plasma levels declined with fasting and rose after feeding. With the use of the value obtained on day 3, 1200 at the end of fasting as base, one-way ANOVA detected that the postprandial values on days 1 and 3 were higher than the bases in both groups, but the differences were of greater statistical significance in the HD patients; $P$ values 0.03 to 0.001 in the controls and 0.002 to $<0.0001$ in the HD patients. Table 4 summarizes PYY kinetics showing one single elimination half life of similar value in the two groups. Its secretory capacity, repre-

Table 3. Leptin kinetics

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>HD</th>
<th></th>
<th>Male</th>
<th>Female</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>$t_{1/2a}$, h</td>
<td>15 ± 1.7</td>
<td>17 ± 4.9</td>
<td>0.53</td>
<td>14 ± 1.2</td>
<td>20 ± 6.0</td>
<td>0.59</td>
</tr>
<tr>
<td>$t_{1/2b}$, h</td>
<td>26 ± 5.8</td>
<td>29 ± 6.1</td>
<td>0.85</td>
<td>32 ± 7.2</td>
<td>23 ± 3.4</td>
<td>0.70</td>
</tr>
<tr>
<td>$C_{\text{max}}$, ng/ml</td>
<td>16 ± 4.3</td>
<td>27 ± 7.2</td>
<td>0.28</td>
<td>13 ± 3.4</td>
<td>34 ± 7.8</td>
<td>0.02</td>
</tr>
<tr>
<td>$C_{\text{min}}$, ng/ml</td>
<td>5 ± 2.1</td>
<td>8 ± 4.4</td>
<td>0.79</td>
<td>3 ± 0.8</td>
<td>12 ± 5.2</td>
<td>0.02</td>
</tr>
<tr>
<td>AUC, ng·h·ml$^{-1}$</td>
<td>233 ± 72</td>
<td>382 ± 108</td>
<td>0.21</td>
<td>183 ± 45</td>
<td>456 ± 117</td>
<td>0.03</td>
</tr>
</tbody>
</table>

Values are means ± SE. Noncompartmental approaches were used for the kinetic analysis. Disappearance rate: $t_{1/2a}$ from day 2, 0300, to day 2, 2400; and $t_{1/2b}$, day 3, 2400, to day 3, 1200. Area under the curve (AUC), from day 3, 1200, to day 4, 0300, from the end of fasting to the peak after refeeding was the surrogate for appearance rate. Maximal and minimal plasma concentrations ($C_{\text{max}}$ and $C_{\text{min}}$) were determined from visual inspection of the concentration-time curves during the refeeding period. $P$ values were derived from Student’s $t$-test.
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Fig. 3. Gut-derived hormone profile. Fifteen blood samples were obtained from each study subject (○, control; ●, HD). A: peptide YY (PYY) was persistently fourfold higher in HD patients. *P < 0.0001 comparing controls vs. HD patients at the same time points by Student’s t-test. The dip in plasma level between 0800 and 1000 on day 3 in HD patients suggested removal by hemodialysis. During refeeding, postprandial rise appeared more robust in the HD group and area under the curve analysis from the end of fasting to postmeal peak confirmed significantly higher value in the HD patients (P = 0.0004). Repeated-measures ANOVA was used to compare the day 3, 1200 value (x inside the circle) to all other values during the entire study period within each group. †P = 0.01 to 0.001 for the controls; ‡P = 0.002 to <0.0001 for the HD patients. The postmeal rises were of greater magnitude in the HD group. B: acylated-ghrelin was lower in the HD patients, and the preprandial rises, especially during refeeding, were blunted. *P = 0.05 to 0.003, comparing control vs. HD at the same time points by Student’s t-test. Value from day 3, 1400 (x inside circle) was used as base and compared with all other values within the same group. †P = 0.03 to 0.001 for the controls; ‡P = 0.05 to 0.02 for the HD patients. The height of preprandial rise was diminished in the HD group. The dip between 0800 and 1000 on day 3 in the HD group suggested removal by dialysis. C: plasma glucagon-like peptide-1 7–36 (GLP-17–36) levels were comparable between the 2 groups. During refeeding, its postprandial rise appeared more prominent in the HD group, but area under the curve, from end of fasting to peak following refeeding, was not different.

Table 4. PYY kinetics

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>HD</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>t1/2, h</td>
<td>32 ± 6</td>
<td>41 ± 6</td>
<td>0.33</td>
</tr>
<tr>
<td>Cmax, pg/ml</td>
<td>167 ± 22</td>
<td>446 ± 101</td>
<td>0.0005</td>
</tr>
<tr>
<td>Cmin, pg/ml</td>
<td>64 ± 7</td>
<td>221 ± 15</td>
<td>0.0004</td>
</tr>
<tr>
<td>AUC, pg·h·ml⁻¹</td>
<td>992 ± 123</td>
<td>2,800 ± 192</td>
<td>0.0004</td>
</tr>
</tbody>
</table>

Values are means ± SE. Noncompartmental approach was used to estimate pharmacokinetic parameters. The t1/2 was obtained from the decline of plasma levels from day 1, 2000 to day 3, 0800. We did not use the day 3, 1200 value because of its removal by HD from 0800 to 1000. Appearance rate was calculated from the rise in plasma concentration from the end of fasting to the peak after refeeding as represented by AUC from day 3, 1200 to day 3, 2000. Cmax and Cmin were determined from visual inspection of the concentration-time curves during refeeding. P values derived from Student’s t-test.
Table 6 summarizes the data on protein homeostasis. In the HD patients, protein intake at baseline was lower, but in the refeeding period when ad libitum eating was allowed, protein intake rose to above that of the controls. Protein output, derived from nNPA, was not different between the two study groups. Protein balance was markedly negative during fasting and neutral during baseline and refeeding in both groups. The control group showed a significant reduction of protein output during fasting while the HD group did not. Protein output and protein balance were not different between the two groups during any of the three study periods.

DISCUSSION

Endocrine dysfunction is prevalent in patients with end-stage renal diseases (39). In the current study, we describe hormonal abnormalities involved in the regulation of energy homeostasis, including high leptin, elevated PYY, low ghrelin, and increased NPY.

High plasma leptin in CKD patients is generally attributed to impaired degradation (47, 60, 71); the current data suggest that the high level was, in part, due to increased production. In fasting, leptin degradation, calculated from a single exponential decay, was similar between the two groups (Table 3). Although the greater leptin production rate in the HD patients, covering a period from the end of fasting to the peak after refeeding, failed to reach statistical significance by the Student t-test, multifactorial stepwise forward and reverse linear regression analysis demonstrated significant associations of the AUC with HD status, sex, and body fat. Hence, the failure to detect a difference between the two groups by conventional...
HORMONAL PROFILE DURING FASTING AND FEEDING IN HEMODIALYSIS PATIENTS

Table 5. Energy intake and expenditure

<table>
<thead>
<tr>
<th></th>
<th>Basal</th>
<th>Fasting</th>
<th>Refeeding</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intake, kcal·kg⁻¹·day⁻¹</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>29 ± 2†</td>
<td>0 ± 0‡</td>
<td>25 ± 3</td>
</tr>
<tr>
<td>HD</td>
<td>26 ± 2*</td>
<td>0.2 ± 0.2‡</td>
<td>27 ± 3</td>
</tr>
<tr>
<td>P</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>RMR, kcal·kg⁻¹·day⁻¹</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>19 ± 0.8†</td>
<td>19 ± 0.9‡</td>
<td>23 ± 1.4</td>
</tr>
<tr>
<td>HD</td>
<td>19 ± 0.9</td>
<td>18 ± 2.0</td>
<td>24 ± 1.5</td>
</tr>
<tr>
<td>P</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
</tbody>
</table>

Values are means ± SE. Energy intake was taken from the record of food consumption at the General Clinical Research Center and expenditure, measured as resting metabolic rate (RMR). Basal RMR was obtained after an overnight fast, fasting RMR was obtained after a 36-h fast, and refeeding was obtained half an hour after lunch on day 3. P values, comparing control (C) vs. HD, were derived from Student’s t-test. *, †, ‡Statistically significant differences comparing, respectively, basal vs. fasting, basal vs. refeeding, and refeeding vs. fasting by ANOVA within each group.

Table 6. Protein homeostasis

<table>
<thead>
<tr>
<th></th>
<th>Basal</th>
<th>Fasting</th>
<th>Refeeding</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intake, g·kg⁻¹·day⁻¹</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>1.07 ± 0.1†</td>
<td>0 ± 0‡</td>
<td>0.80 ± 0.09</td>
</tr>
<tr>
<td>HD</td>
<td>0.82 ± 0.07*</td>
<td>0.01 ± 0.01‡</td>
<td>0.94 ± 0.08</td>
</tr>
<tr>
<td>P</td>
<td>0.04</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Output, g·kg⁻¹·day⁻¹</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>0.96 ± 0.1*</td>
<td>0.60 ± 0.04</td>
<td>0.84 ± 0.1</td>
</tr>
<tr>
<td>HD</td>
<td>0.79 ± 0.03</td>
<td>0.76 ± 0.06</td>
<td>0.84 ± 0.05</td>
</tr>
<tr>
<td>P</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Balance, g·kg⁻¹·day⁻¹</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>0.12 ± 0.05*</td>
<td>-0.60 ± 0.04‡</td>
<td>-0.04 ± 0.06</td>
</tr>
<tr>
<td>HD</td>
<td>0.03 ± 0.12*</td>
<td>-0.76 ± 0.07‡</td>
<td>0.10 ± 0.08</td>
</tr>
<tr>
<td>P</td>
<td>NS</td>
<td>NS</td>
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</tr>
</tbody>
</table>

Values are presented as means ± SE. Intake was taken form the record of food consumption, and output in the controls was derived from 24-h urine urea nitrogen output and in HD patients by urea kinetics. Balance = intake − output. P values were derived from Student’s t-test comparing control vs. HD. *, †, ‡Statistically significant differences comparing, respectively, basal vs. fasting, basal vs. refeeding, and refeeding vs. fasting by ANOVA within each group.
hormone profile. These underscore the complexity of mammalian appetite regulation which is multilayered and redundant. The absence of anorexia could be due to hormone resistance when the receptors are unable to respond normally to the signal, as in the case of obesity when circulating leptin is high. Impaired parasympathetic activity due to vagus nerve dysfunction could explain PYY resistance. The vagus nerve, in addition to its inhibitory effect on PYY secretion, is also essential in mediating its anorexigenic effect. Koda et al. (30) found that abdominal vagotomy abolished the PYY-induced decline in feeding. While intravenous PYY administration to control rats resulted in a significant reduction in food consumption compared with saline, PYY given to vagotomized rats did not reduce food intake. PYY-induced Fox expression in the arcuate nucleus was also abolished by vagotomy, suggesting that the satiety message needs the afferent vagus fibers to reach the nucleus tractus solitarius. Likewise, the vagus nerve is needed to mediate the orexigenic effect of ghrelin. In rats, vagotomy abolishes ghrelin-stimulated feeding (17); in humans, ghrelin does not stimulate appetite in those who had surgical procedures involving vagotomy (58). Thus to the extent that HD patients have suppressed parasympathetic function (1, 55, 68), they may have blunted anorexigenic and orexigenic response to PYY and ghrelin, respectively.

Our HD patients also did not have energy wasting or excessive protein catabolism, findings consistent with our previous work (40, 41) showing adequate protein conservation. The dietary intake in our patients, 26 kcal·kg⁻¹·day⁻¹ for energy and 0.8 g·kg⁻¹·day⁻¹ for protein, was substantially below that recommended by NKF-K/DOQI guidelines, 35 kcal·kg⁻¹·day⁻¹ (for patients <60 yr) and 1.2 g·kg⁻¹·day⁻¹, respectively (35). In the HEMO Study, the mean intake were comparably low, 23 kcal·kg⁻¹·day⁻¹ and 0.93 g·kg⁻¹·day⁻¹, respectively. Compared with the HEMO study, our patients were younger, 41 vs. 58 yr; had greater body mass index, 28 vs. 25; higher serum albumin, 4.2 vs. 3.6 g/dl; and were devoid of comorbid illnesses (56, 57). Thus, when end-stage renal disease patients are well dialyzed and suffer no comorbid illnesses, they can maintain good nutrition on a diet consisting of fewer calories and lesser protein than currently recommended.

Serendipitously, we noted that the abnormalities uncovered in this study have other functions independent of energy homeostasis. Elevated leptin could increase sympathetic nerve activity (23, 63) and may impair baroreceptor sensitivity (3). PYY is a powerful vasoconstrictor. Its injection into the superior mesenteric arteries of cats caused intestinal ischemia (43). In humans, PYY injection into the brachial artery resulted in a dose-dependent decline in forearm blood flow (52). NPY likewise has a vasoconstrictive effect, and it enhances the vascular effect of norepinephrine and angiotensin II (22, 50). Recently, NPY was also shown to induce arterial occlusion following balloon angioplasty in rats (37), and Zoccali et al. (73) reported that high plasma NPY predicts adverse cardiovascular events in HD patients.

Because ghrelin has a potent vasodilatation property, its diminished level also predisposes HD patients to vasoconstriction. Nagaya et al. (49) found that intravenous injection of ghrelin to healthy men resulted in a decrease in mean arterial blood pressure without any change in heart rate, an increase in stroke volume because of reduced cardiac after load. Patients with metabolic syndrome have reduced plasma ghrelin, and they showed enhanced sensitivity to endothelin-induced vasoconstriction and impaired nitric oxide-dependant vasodilatation; these abnormalities were corrected by ghrelin infusion (66). Therapeutically, ghrelin infusion not only increased food intake, but also lowered blood pressure in CKD patients (69).

In summary, endocrine evaluation of otherwise healthy HD patients showed normal appetite and metabolism but exhibited marked abnormalities in hormones involved in the regulation of appetite and energy balance. We attribute these seemingly paradoxical findings to vagal dysfunction, impaired parasympathetic activity, a phenomenon well-described in CKD patients. Most importantly, these hormonal abnormalities may heighten sympathetic activity, augment vasoconstriction, and impair vasodilatation, all of which, in the long run, pose adverse effects on the integrity of the cardiovascular system in the HD patients.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

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