Association of Plasma Des-acyl Ghrelin Levels with CKD

Rohit K. Gupta, Tamil Kuppusamy,* James T. Patrie,† Bruce Gaylinn,§ Jianhua Liu,§ Michael O. Thorner,§ and Warren K. Bolton**

Summary

Background and objectives There are no effective therapies for malnutrition in CKD/ESRD patients. This study hypothesized that ghrelin, an endogenous orexigenic hormone, would correlate with renal function and might suggest therapeutic interventions for CKD/ESRD malnutrition.

Design, setting, participants, & measurements Fifty-one CKD and 15 hemodialysis patients were enrolled. Acyl ghrelin (AG) and des-acyl ghrelin (DG) were determined using separate two-site–specific assays. Leptin, insulin, growth hormone, insulin-link growth factor-1, C-reactive protein, TNF-α, and IL-6 were also measured.

Results Univariate correlation analyses showed that CKD stage was highly, positively correlated with the levels of preprandial and postprandial DG and positively correlated with TNF-α, IL-6, leptin, and age. Multivariate partial-correlation analyses showed that CKD was independently associated with the proportion of preprandial and postprandial DG, whereas TNF-α, IL-6, leptin, insulin, and age were not independently associated with either. Geometric mean (GM) preprandial and postprandial AG were comparable between CKD stages $\leq$2 and $>$2, whereas GM preprandial DG and postprandial DG were 1.95-fold and 2.17-fold greater, respectively, for CKD stage $>$2 versus stage $\leq$2. DG was the dominant form of ghrelin preprandially and postprandially for both CKD stages $\leq$2 and $>$2. Dialysis had no effect on AG, but reduced DG by 73% to levels even lower (GM 48.7 pg/ml) than those seen postprandially in CKD stage $\leq$2 patients (GM 77.0 pg/ml).

Conclusions This study shows a strong and independent correlation of DG with CKD stage. Postprandial suppression of ghrelin is impaired with reduced renal function. Hemodialysis selectively removes DG but not AG.

Clin J Am Soc Nephrol 8: 1098–1105, 2013. doi: 10.2215/CJN.09170912

Division of *Nephrology, Department of Medicine, †Division of Biostatistics, Department of Public Health Sciences, §Division of Endocrinology, Department of Medicine, University of Virginia Health Sciences Center, Charlottesville, Virginia

Correspondence: Dr. W. Kline Bolton, Division of Nephrology, University of Virginia Health Sciences Center, Box 800133, Charlottesville, VA 22908. Email: wkb5s@virginia.edu

Introduction

Malnutrition/protein-energy wasting is a major cause of morbidity and mortality for patients suffering from CKD. Approximately 20%–50% of patients with CKD suffer from malnutrition, and 70%–75% of patients with ESRD have protein-energy wasting (1–3).

As patients develop renal failure, they lose their taste for food and decrease nutritional intake (4). Furthermore, increased catabolism or decreased anabolism can lead to cachexia. Hormones such as insulin, growth hormone (GH) and insulin-like growth factor-1 (IGF-1) stimulate protein synthesis, whereas a decrease in these mediators may cause a decline in anabolism resulting in malnutrition. Inflammatory cytokines, such as TNF-α, C-reactive protein (CRP), and IL-6, have been implicated in the increased protein catabolism that characterizes muscle wasting of uremia, and appetite suppression by leptin has also been implicated (5–11).

Ghrelin is an orexigenic hormone that may be involved in the malnutrition of uremia (12–14). It is a 28 amino acid peptide that acts at the GH secretagogue receptor (GHSR) to stimulate appetite, carbohydrate utilization, and GH release. Conflicting reports of the levels of total ghrelin (TG) and acyl ghrelin (AG) in patients with renal dysfunction have been difficult to interpret and reconcile (15–21). Most reports have used single antibody ghrelin assays that recognize an epitope found only on AG or alternatively, an epitope common to both AG and des-acyl ghrelin (DG). Unfortunately, these assays also detect inactive ghrelin fragments. Thus, TG as reported in the literature actually includes AG+DG + ghrelin fragments. We have developed assays to measure full-length AG and DG but not fragments (22,23). We hypothesized that ghrelin levels would correlate with renal function and might suggest therapeutic interventions for CKD/ESRD malnutrition. The purpose of our study was to examine this hypothesis with our assays.

Materials and Methods

Study Participants

There were two groups of patients. Group 1 had CKD not requiring dialysis. Group 2 patients had ESRD and were receiving thrice-weekly hemodialysis. Participants were aged $\geq$18 years and provided informed consent before enrollment. The study was approved by our institutional review board and complied with the Declaration of Helsinki guidelines.

CKD Patients

Patients seen in our Kidney Center Clinic with CKD from each of the five CKD stages were recruited. The estimated GFR (eGFR) was calculated using the Modified Diet in Renal Disease study equation. Individuals were screened in the clinic and were then screened for inclusion/exclusion. They then were contacted by phone to see if they would be willing to participate. This process was followed until there were 10 individuals in each CKD stage. One individual did not want to participate and then changed their mind. Rather than discourage that individual, we included the patient and one group thus had 11 individuals. Patient characteristics are provided in Table 1. After an overnight fast, plasma samples were obtained at time zero (T=0). The following assays were performed: DG, AG, IL-6, TNFα, GH, insulin, IGF-1, CRP, albumin, and leptin. After the fasting samples were obtained, a standard breakfast was provided. The meal consisted of 55% carbohydrates, 30% fat, and 15% protein. It contained 500 kcal with 500 mg of sodium, 450 mg of potassium, 300 mg of phosphorus, and 240 ml of free fluids. A body mass index was calculated, and a subjective global assessment (SGA) was performed by a licensed nutritionist (6). One hour after completion of the meal (T=1), repeat AG and DG samples were drawn.

Hemodialysis Patients

Fifteen hemodialysis patients participated. Patients were screened for inclusion and exclusion criteria and then approached about their participation in the study. Patient characteristics are presented in Table 2. Patients arrived at the dialysis center in a fasting state for initial plasma samples (T=0), and then received the standard breakfast and assessment noted above except an SGA was not obtained. Ghrelin levels were measured at 1 hour after the meal (T=1), and dialysis was initiated. A postdialysis ghrelin sample was obtained immediately after each patient received their standard dialysis session (posthemodialysis) (T=4).

Assays for Hormones and Cytokines

The following assays were performed: GH, IGF-1, insulin, hsCRP, IL-6, TNF-α, and leptin. Please see the Supplemental Material for details of the assays.

Table 1. Demographics and patient characteristics for the CKD patients, determined at the prestudy clinic visit

Variable	Overall (n=46)	CKD Stage 1 (n=9)	CKD Stage 2 (n=7)	CKD Stage 3 (n=11)	CKD Stage 4 (n=10)	CKD Stage 5 (n=9)
Demographics						
Female sex	27 (58.7)	6 (66.7)	4 (57.1)	9 (81.8)	6 (60.0)	2 (22.2)
Race (Caucasian)	29 (63.0)	6 (66.7)	5 (71.4)	7 (63.6)	7 (70.0)	4 (44.4)
Age (yr)	57.2 [39.6, 65.1]	39.1 [36.9, 51.8]	48.6 [34.4, 51.7]	56.7 [44.9, 56.8]	61.3 [58.0, 65.8]	62.2 [54.5,68,2]
Anthropometrics						
BMI	30.5 [27.0, 36.5]	30.0 [30.0, 33.3]	27 [24.5, 31.0]	32.0 [30.0, 38.0]	29.0 [27.0, 37.0]	31.0 [28.0, 37.0]
Laboratory values						
Na (mmol/L)	139.0 [137.2, 140.0]	139.0 [137.7, 139.0]	140.0 [139.0, 141.0]	138.0 [136.0, 139.0]	138.5 [138.0, 140.0]	139.0 [136.0, 140.2]
K (mmol/L)	3.9 [3.8, 4.1]	4.0 [4.0, 4.1]	4.0 [3.7, 4.3]	4.0 [3.7, 4.3]	4.4 [3.8, 4.8]	4.3 [4.1, 4.7]
Cl (mmol/L)	104.0 [103.0, 106.0]	104.0 [103.0, 105.2]	105.0 [103.0, 106.0]	103.0 [102.0, 106.0]	104.0 [103.0, 107.0]	105.0 [102.8, 106.2]
CO_2 (mmol/L)	24.0 [22.0, 26.0]	25.0 [22.7, 26.0]	26.0 [24.5, 28.0]	24.0 [22.0, 26.0]	23.5 [22.0, 24.7]	20.0 [18.5, 22.5]
BUN (mg/dl)	26.0 [17.0, 43.5]	12.0 [9.2, 15.5]	17.0 [14.0, 20.0]	23.0 [19.0, 27.0]	43.0 [37.0, 53.7]	60.0 [49.5, 78.2]
Cr (mg/dl)	1.5 [1.0, 3.1]	0.8 [0.8, 0.8]	1.0 [0.9, 1.2]	1.5 [1.3, 1.5]	2.9 [2.5, 3.3]	5.2 [4.4, 6.1]
Ca (mg/dl)	9.2 [9.0, 9.5]	9.0 [8.9, 9.1]	9.2 [9.0, 9.4]	9.3 [9.2, 9.5]	9.2 [9.1, 9.7]	9.0 [8.3, 9.1]
Phosphate (mg/dl)	3.1 [2.8, 3.5]	2.7 [2.5, 3.0]	3.4 [2.9, 3.4]	3.0 [2.9, 3.4]	3.0 [2.8, 3.5]	3.7 [2.9, 4.5]
Hgb (g/dl)	12.9 [12.2, 13.9]	14.0 [13.9, 14.5]	14.4 [14.2, 14,8]	12.3 [12.0, 13.9]	12.8 [12.5, 13.3]	12.3 [11.6, 12.8]
Vital signs						
SBP (mmHg)	138.5 [124.2, 158.7]	126 [122.0, 145.0]	141.0 [123.0, 156.0]	142.0 [132.0, 165.5]	136.0 [125.7, 150.7]	135.0 [126.0, 192.0]
DBP (mmHg)	78.0 [70.0, 87.7]	73.0 [75.5, 89.5]	78.9 [75.5, 89.5]	70.0 [69,0.0, 91.0]	74.0 [69.0, 84.7]	82.0 [78.0, 83.0]
Pulse rate (beats/min)	74.0 [64.0, 80.0]	77.0 [64.5, 83.5]	78.0 [59.0, 77.0]	72.0 [68,5, 78.2]	74.0 [68.5, 78.2]	67.0 [60.0, 81.0]

Categorical variables are summarized by the frequency (%). Continuous variables are summarized by the median [interquartile range] of the measurement distribution. BMI, body mass index; Na, sodium; K, potassium; Cl, chloride; CO_2, bicarbonate; Cr, creatinine; Ca, calcium; Hgb, hemoglobin; SBP, systolic BP; DBP, diastolic BP.

Table 2. Demographics and patient characteristics for the 15 hemodialysis patients

Variable	Summary Measure
Demographics	
Female sex	5 (33.3)
Race (Caucasian, black)	5 (33.3), 9 (60.0)
Age (yr)	50.0 [46.0, 62.0]
Anthropometrics	
BMI	22.5 [21.9, 28.0]
Medication use (%)	
Insulin	2.0 (13.3)
Statins	6 (40.0)
Dialysis	
Dialysis time (h)	3.3 [3.0, 4.0]
Average monthly fluid removal per treatment (L)	3.4 [2.9, 3.9]
Fluid removed during session (ml)	3321.0 [2551.0, 4952.5]
Kt/V	1.6 [1.5, 1.8]
URR	0.7 [0.7, 0.8]
Laboratory values	
Na (mmol/L)	137.0 [134.5, 138.0]
K (mmol/L)	4.7 [4.4, 5.1]
Cl (mmol/L)	95.0 [93.5, 98.0]
CO_2 (mmol/L)	22.0 [19.0, 26.0]
Ca (mmol/L)	9.2 [8.4, 10.1]
Glucose (mg/dl)	114 [98, 155]
Phosphate (mg/dl)	5.9 [4.9, 6.6]
HCT (%)	37.0 [34.5, 38.7]
Hgb (g/dl)	11.8 [11.5, 12.5]
Albumin (g/dl)	3.8 [3.2, 4.1]
Predialysis BUN (mg/dl)	47.0 [45.0, 50.0]
Postdialysis BUN (mg/dl)	13.0 [7.5, 11.5]
Cr (mg/dl)	9.2 [7.5, 11.5]
eGFR (ml/min per 1.73 m^2)	6.5 [5.0, 8.2]
PTH (pg/ml)	122.4 [88.3, 224.2]

Categorical variables are summarized by the frequency (%). Continuous variables summarized by the median and [interquartile range] of the measurement distribution. BMI, body mass index; Kt/V, dialysis adequacy; URR, urea reduction ratio; Na, sodium; K, potassium; Cl, chloride; CO_2, bicarbonate; Ca, calcium; HCT, hematocrit; Hgb, hemoglobin; Cr, creatinine; PTH, parathyroid hormone.

Assay for Ghrelin

We used two separate two-site sandwich assays, one specific for AG and one for DG. These assays do not measure ghrelin fragments and have superior specificity for AG and DG determination (22,23).

Sample Collection for Ghrelin Assay

Three-milliliter blood samples were collected by syringe and immediately added to chilled EDTA Vacutainer tubes preloaded with aminoethyl-benzenesulfonylfluoride (AEBSF) to give a 4 mM final concentration. The tubes were kept on ice and spun immediately in a chilled centrifuge for 10 minutes to separate the plasma. The plasma supernatant was collected, acidified with 200 μl of 1 N HCI/1000 μl plasma, mixed, and then frozen in aliquots at −20°C.

AG Sandwich Assay

Plates (384-well Maxisorb; Nunc, Roskilde, Denmark) were coated with acyl-specific antiserum at 1 μg/ml overnight. The plate was then blocked, washed, and loaded with 25 μl/well wetting/neutralization buffer (0.5 M phosphate buffer with 1% BSA, pH 7.4) and 25 μl/well ghrelin standards or unknown samples and incubated overnight at 4°C. The washed plate was then incubated for 1 hour with the biotinylated C-terminal ghrelin antiserum in blocking buffer and then for 30 minutes with the streptavidin-poly-HRP80 (RDI Fitzgerald, Concord, MS). Finally, the plate was detected with the florescent substrate Amplex Red (Molecular Probes, Eugene, OR). Fluorescence was read using excitation/emission wavelengths of 535/590 nm (Tecan Genios plate reader; Phenix Research, Hayward, CA). All unknowns were run in duplicate, and all samples for each admission of each individual were run on the same plate. Standards were made up in acid/AEBSF-treated C18 (Empore; 3M, St. Paul, MN) stripped plasma. The assay sensitivity was 6.7 pg/ml with an intra-assay coefficient of variation (CV) of 9.1% at 30 pg/ml, 12.6% at 100 pg/ml, and 16.8% at 300 pg/ml. The inter-assay CV was 17.8% at 50 pg/ml.

DG Sandwich Assay

The protocol for DG assay follows that used for the ghrelin sandwich assay with the substitution of affinity-purified C-terminal ghrelin antiserum for the capture step and biotinylated N-terminal DG-specific monoclonal antiserum as the reporter. All other steps are unchanged. The assay sensitivity was 4.6 pg/ml with an intra-assay CV of 12.5% at 50 pg/ml, 10.7% at 150 pg/ml, and 18.0% at 500 pg/ml. The inter-assay CV was 20.8% at 30 pg/ml.

Statistical Analyses

Patient characteristics were summarized by frequencies and by percentages for categorical variables, and by the median (interquartile range) for continuous variables.

Bivariate correlations were estimated *via* the nonparametric Spearman rank correlation coefficient (r_s). Multivariate partial-correlations were estimated *via* the nonparametric Spearman rank partial-correlation coefficient (r_{sp}). All tests for bivariate and partial association were two sided and a $P \leq 0.05$ decision rule was utilized as the rejection criterion.

Preprandial and postprandial AG as well as DG concentrations were analyzed *via* repeated-measures ANOVA on the natural logarithmic scale. Comparisons of mean ghrelin concentration were made on logarithmic scale and then converted back to the original scale of measure *via* the antilog transformation. Hypothesis tests were two sided and a $P \leq 0.05$ decision rule was utilized as the rejection criterion. Results are expressed in terms of the geometric mean (GM) and the ratio of GM. Like the arithmetic mean and median, the GM is a statistic that is utilized to estimate the central location of the measurement distribution.

SAS software (version 9.2; SAS Institute Inc., Cary, NC) and Spotfire S Plus software (version 8.2; TIBCO Inc., Seattle, WA) were used to conduct the statistical analyses.

Results

CKD

Patient Characteristics. There were 10 participants in each of the five CKD categories except for CKD stage 3, which had 11 individuals. Five participants had inadequate samples for the assays; thus, data from 46 individuals were available for analysis. There were 19 men (41%) and 27 women (59%), of whom, 17 were African American (37%) and 29 were Caucasian (63%). Ages ranged from 21 to 82 years (median age 57 years; interquartile range, 40–65 years). There were no differences in body mass index or SGA. There were also no differences in appetite, time or quantity of food consumed at the study session, or evidence of malnutrition (Supplemental Tables 1–3). As selected, the eGFR was lower with the higher stage of CKD.

Correlation Analyses. Univariate correlation analyses showed that CKD stage was positively correlated with preprandial and postprandial DG (r_s=0.33 and 0.34, respectively; P=0.03 and 0.02, respectively), but not with preprandial and postprandial AG. CKD stage was also highly, positively correlated with the proportion of preprandial and the proportion of postprandial DG (r_s=0.61 and r_s=055, respectively; $P<0.001$ for both). Similarly, univariate correlation analyses showed CKD stage was positively correlated with TNF-α, IL-6, leptin, and age (r_s=0.33, 0.40, and 0.41, respectively; $P<0.05$ for all) (Table 3). Multivariate partial-correlation analyses showed that CKD was independently associated with the proportion of preprandial and postprandial DG (r_{sp}=0.55 and 0.43, respectively; $P<0.01$ for both), whereas TNF-α, IL-6, leptin, insulin, and age were not independently correlated with either of these two proportions (Table 4). Even with CKD stage removed from the set of partial correlates, TNF-α, IL-6, leptin, insulin, and age were not independently correlated with the proportion of either preprandial or postprandial DG (Supplemental Table 4).

Repeated-Measures Analyses. GM preprandial and GM postprandial AG were comparable between CKD stage ≤2 patients and CKD stage >2 patients, whereas GM preprandial DG and GM postprandial DG were 1.95-fold (95% confidence interval [95% CI], 1.16 to 3.26; P=0.01) and 2.17-fold (95% CI, 1.16 to 3.26; P=0.004) greater, respectively, for CKD stage >2 patients compared with CKD stage ≤2 patients. Compared with AG, DG was the dominant preprandial and postprandial ghrelin form for both CKD stage ≤2 patients ($P<0.001$ for both), and CKD stage >2 patients ($P<0.001$ for both). GM postprandial DG was reduced by 26% (95% CI, 5% to 43%; P=0.02) and by 18% (95% CI, 1% to 32%; P=0.03), respectively, for CKD stage ≤2 patients and CKD stage >2 patients compared with GM preprandial DG. GM postprandial AG was comparable with GM preprandial AG for both CKD stage ≤2 and CKD stage >2 patients (Figure 1).

ESRD

The levels of preprandial, postprandial, and postdialysis ghrelin for the ESRD patients are illustrated in Figure 2. There was no decrease in AG postprandially, nor any changes in AG resultant from dialysis. DG actually increased slightly postprandially but the magnitude of the increase was not statistically significant. However, a standard dialysis treatment resulted in a significant decrease in DG of 73% (95% CI, 0.50% to 85%; $P<0.001$). Dialysis therapy resulted in reduction of DG to levels (GM 48.7 pg/ml; 95% CI, 29.5 to 80.2 pg/ml) even lower than those seen postprandially in CKD stage 1 (n=2 patients; GM 77.0 pg/ml; 95% CI, 51.2 to 117.9 pg/ml). The other correlations in dialysis patients between various significant parameters and ghrelin are presented in Supplemental Table 5. Both preprandial and postprandial DG were negatively correlated with serum albumin levels (r_s=−0.64 and −0.59, respectively; P=0.01 and 0.02, respectively) in contrast there was no correlation between AG and serum albumin levels. Preprandial GH correlated negatively with IGF-1, insulin, and leptin (r_s=−0.65, −0.71, and −0.67, respectively; $P<0.01$ for all). IGF-1 correlated with insulin, leptin, and albumin (r_s=0.79, 0.59, and 0.65, respectively; $P<0.01$, 0.02, and 0.01, respectively). Insulin was correlated positively with leptin and albumin (r_s=0.81 and 0.59, respectively; $P<0.001$ and 0.02, respectively). CRP and IL-6 were positively correlated with each other (r_s=0.53; P=0.04).

Discussion

Our study examined changes in DG and AG in CKD and ESRD using specific and sensitive assays for these hormones (22,23). We demonstrate that levels of DG but not AG increase with declining eGFR, and that ghrelin metabolism or secretion may be altered by uremia. AG response to feeding was blunted in CKD and absent in ESRD participants, as also reported by others (24,25). These results suggest that AG secretion is within the normal range and its conversion to DG is normal, but DG accumulates as its clearance is progressively decreased with declining eGFR. The increment in DG was tightly linked to the decrease in eGFR so that it correlated with CKD stages 3–5 with high accuracy.

Increased levels of TG with decreasing GFR have been reported by others (15–19,21,26) as well as variable removal by dialysis (16,18,21). Some studies reported a decrease in both AG and DG (16,18) by hemodialysis that would not be expected for AG, which is highly bound to large plasma molecules such as lipoprotein (27,28). Other studies report no change in plasma ghrelin levels with hemodialysis (21). These results differ from those reported here with our ghrelin sandwich assay (22,23,29) and likely reflect the use of different assays with different specificities for the ghrelin molecule, and differences in sample preparation and preservation. The method described in our assay preserves the natural ratio between AG and DG without the confounder of measuring ghrelin fragments.

TG normally precipitously decreases postprandially, but the DG/AG ratio is maintained (22). In normal individuals, the ratio of DG to AG is approximately 4:1 with 20% AG, and feeding causes a 2- to 5-fold decrease but the ratio is not changed (22). In long-term fasted (>37 hours) normal individuals, the ratio is approximately 12:1 (*i.e.*, greater DG to AG than fed individuals) (22). We found that this relationship is altered in CKD. The DG/AG ratio is similar to that in long-term fasting individuals, and the decrease with feeding is attenuated relative to normal (22). These changes suggest that altered ghrelin metabolism associated with uremia, favoring DG predominance. This was exaggerated in ESRD, in which there was no significant

Table 3. Univariate correlations with CKD stage

Correlate	Spearman Correlation, r_s (95% CI)	*P* Value
Preprandial DG/(AG+DG)	0.61 (0.38 to 0.77)	<0.001
Postprandial DG/(AG+DG)	0.55 (0.30 to 0.73)	<0.001
Age	0.41 (0.13 to 0.63)	0.01
IL6	0.40 (0.11 to 0.62)	0.01
Postprandial DG	0.34 (0.05 to 0.58)	0.02
Leptin	0.34 (0.04 to 0.58)	0.02
TNFα	0.33 (0.04 to 0.57)	0.02
Preprandial DG	0.31 (0.02 to 0.56)	0.03
Insulin	0.24 (−0.06 to 0.50)	0.11
GH	−0.20 (−0.47 to 0.10)	0.18
Postprandial AG	0.14 (−0.17 to 0.42)	0.36
Preprandial AG	0.11 (−0.19 to 0.40)	0.46
CRP	0.08 (−0.22 to 0.37)	0.59
Albumin	−0.07 (−0.36 to 0.23)	0.65
IGF-1	0.07 (−0.24 to 0.36)	0.66

Univariate Spearman rank correlations for identifying bivariate relationships between CKD stage and AG, DG, IL6, leptin, TNF-α, insulin, GH, CRP, albumin, and IGF-1. 95% CI, 95% confidence interval; AG, acyl grehlin; DG, des-acyl ghrelin; GH, growth hormone; CRP, C-reactive protein; IGF-1, insulin-like growth factor-1.

change in either DG or AG postprandially in dialysis patients (Figure 2). Peréz-Fontan *et al.* and Suneja *et al.* likewise reported perturbation in the expected responses to feeding in dialysis patients as noted above (24,25)

Ghrelin levels are increased in states of severe malnutrition and decreased in states of satiety (7,30,31). Ghrelin has potent orexigenic activity and induces an increase in food intake, and muscle mass with a positive change in energy uremic balance in rats (32–34). Ghrelin administered to dialysis patients increased appetite and food intake, induced a sustained positive change in energy balance (35,36) and increased food intake in malnourished patients on peritoneal dialysis (35). Thus, a decrease in AG in CKD could theoretically play a role in the malnutrition observed in many of these patients. However, we observed no change in AG levels as eGFR declined.

We did demonstrate that DG increased with decreasing eGFR, as described by others (16,37). DG has been reported to be the "inactive" degradation product of AG but recent studies suggest that DG may itself have pleiotropic biologic activities, including opposing actions to AG (38–43). In normal individuals, DG and AG levels decrease in parallel on feeding, but the ratio of DG/AG remains constant (22). In our studies in CKD, the decrease in postprandial levels was blunted, but the ratio of DG/AG was maintained, and there was actually an increase in DG postprandially in dialysis patients. Others have reported that AG metabolism was altered in dialysis patients, but did not report data on DG (24,25). Our findings suggest that not only is DG increased as eGFR declines, but that abnormalities in the response of the ghrelin axis to feeding occur as CKD develops. Increasing evidence implicates a role of DG in the appetite changes of uremia. Administration of DG to normal animals decreased food intake and gastric emptying (44), and increased anorexigenic transcripts in the hypothalamus (44,45). Mice overexpressing DG had decreased body weight, fat pad mass, and growth. Thus, elevated levels of DG could be involved in the pathogenesis of anorexia of uremia. The reduction in DG by dialysis could play a major role in improving the nutritional status in uremia, and might be further augmented by more frequent or continuous therapies. Of note, transplanted patients have a return of TG levels to normal post-transplant (46).

Inflammatory anorexic cytokines are increased in many patients with CKD. Elevation of serum TNFα, IL-6, CRP, and leptin have all been implicated in the genesis of the cachexia of uremia (8,11,47). Our studies demonstrated higher levels of TNFα, IL-6, and leptin with lower eGFR. After adjusting for CKD, there was no association between DG and the cytokines, suggesting that DG may have an independent role in promoting the malnutrition/protein-energy wasting of CKD and ESRD. AG inhibits leptin and proinflammatory anorectic cytokine expression by monocytes and T cells, including IL-1β, IL-6, and TNF-α *in vitro* and demonstrates anti-inflammatory effects in a murine endotoxemia model (48). Thus, the increasing DG levels

Table 4. Multivariate correlations of proportion of DG

Dependent Variable →	Proportion of Preprandial Ghrelin in the DG Form [DG/(AG+DG)]		Proportion of Postprandial Ghrelin in the DG Form [DG/(AG+DG)]	
Partial Correlates ↓	Spearman Partial-Correlation, r_{sp} (95% CI)	*P* Value	Spearman Partial-Correlation, r_p (95% CI)	*P* Value
CKD stage	0.55 (0.30 to 0.76)	<0.001	0.43 (0.10 to 0.68)	0.01
Age	0.04 (−0.35 to 0.36)	0.79	0.04 (−0.23 to 0.35)	0.78
IL6	0.08 (−0.25 to 0.37)	0.62	0.14 (−0.15 to 0.42)	0.38
Leptin	−0.11 (−0.43 to 0.23)	0.47	−0.05 (−0.42 to 0.31)	0.77
TNFα	−0.25 (−0.45 to 0.17)	0.34	0.04 (−0.26 to 0.34)	0.82
Insulin	−0.04 (−0.41 to 0.31)	0.81	0.04 (−0.27 to 0.38)	0.79

Spearman rank partial-correlations (r_{sp}) for identifying unique bivariate relationships between the proportion of ghrelin in the des-acyl form and CKD stage, age, IL6, leptin, TNFα, and insulin. DG, des-acyl ghrelin; AG, acyl grehlin; 95% CI, 95% confidence interval.

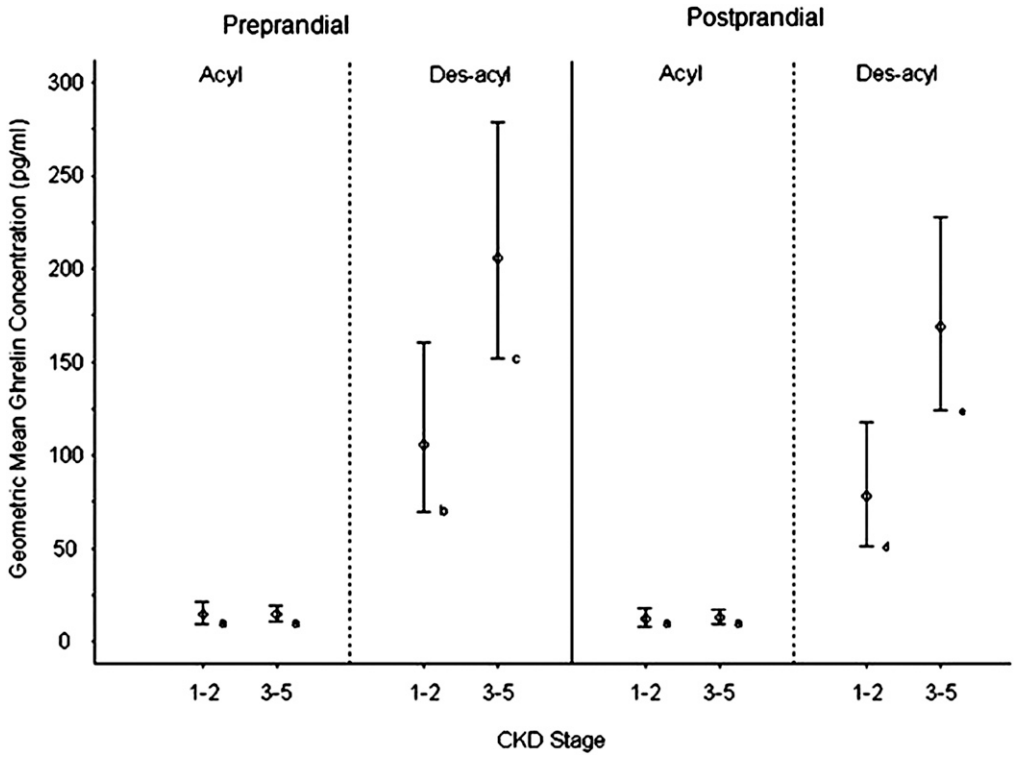

Figure 1. | **Geometric mean ghrelin concentration, by CKD stage and by ghrelin form**. Circles identify the geometric mean of the measurement distribution and the vertical lines identify the range of the geometric mean 95% confidence interval. Geometric means with dissimilar letters signify that the values are statistically significantly different.

in CKD might oppose the effects of AG cytokine inhibition.

Study Strengths and Limitations

Our study utilized assays for AG and DG with greater sensitivity and specificity than previously available, allowing us to measure DG directly. We confirmed some other studies that examined AG and TG and we discovered that DG was most strongly associated with CKD stage, further clarifying the potential role of the ghrelin system in the malnutrition of CKD. The study was limited by small numbers of participants, single time points for samples, and nonrandomized selection of participants. Other markers of malnutrition in CKD may have a different temporal sequence than ghrelin to support the malnutrition state (*e.g.*, peptide YY or neuropeptide Y) (25,49). However, the focus of our study was ghrelin. We showed for the first time that DG is independently dependent on eGFR and that any relationship to inflammatory cytokines is likely directly related to eGFR rather than due to an interaction with ghrelin.

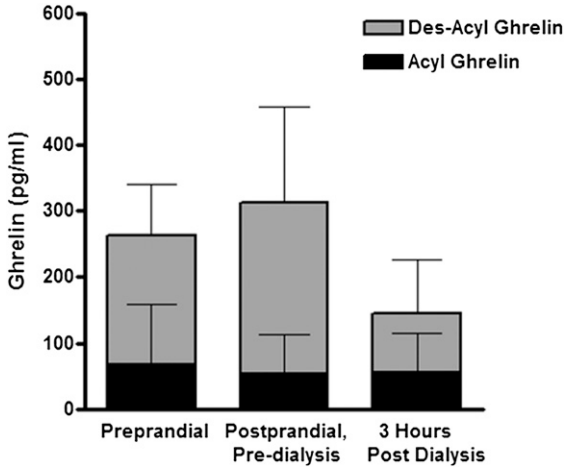

Figure 2. | **Levels of des-acyl ghrelin increased slightly postprandially and were markedly decreased by hemodialysis ($P<0.001$)**. Acyl ghrelin decreased slightly after eating but was not affected by hemodialysis. Error bars indicate the SD.

In conclusion, using sensitive and specific assays for AG and DG, we have demonstrated a tight negative association of DG levels with lower eGFR, impairment of the normal postprandial ghrelin suppression to feeding in CKD and ESRD, and showed that hemodialysis returns DG toward normal. Because orexigenic AG levels were not lower with lower levels of eGFR or decreased by dialysis, a role of DG in the anorexia of uremia is implicated. Additional studies will be required to define the mechanisms of these perturbations of ghrelin and their effect on nutrition and the inflammatory milieu in patients with CKD and ESRD.

Acknowledgments

We appreciate the assistance of Thessa Churillo, nutritionist, and Anita Jacobson, executive secretary.

This work was performed through the Nephrology Clinical Research Center and the University of Virginia General Clinical Research Center (M01 RR0874) and by grants from the National Institutes of Health (R01DK076095 and R21DK077372).

Disclosures

None.

References

1. Ikizler TA: Nutrition, inflammation and chronic kidney disease. *Curr Opin Nephrol Hypertens* 17: 162–167, 2008
2. Muscaritoli M, Molfino A, Bollea MR, Rossi Fanelli F: Malnutrition and wasting in renal disease. *Curr Opin Clin Nutr Metab Care* 12: 378–383, 2009
3. Fouque D, Pelletier S, Mafra D, Chauveau P: Nutrition and chronic kidney disease. *Kidney Int* 80: 348–357, 2011
4. Bossola M, Tazza L, Giungi S, Luciani G: Anorexia in hemodialysis patients: An update. *Kidney Int* 70: 417–422, 2006
5. Stenvinkel P, Pecoits-Filho R, Lindholm B: Leptin, ghrelin, and proinflammatory cytokines: Compounds with nutritional impact in chronic kidney disease? *Adv Ren Replace Ther* 10: 332–345, 2003
6. Steiber AL, Kalantar-Zadeh K, Secker D, McCarthy M, Sehgal A, McCann L: Subjective global assessment in chronic kidney disease: A review. *J Ren Nutr* 14: 191–200, 2004
7. Bologa RM, Levine DM, Parker TS, Cheigh JS, Serur D, Stenzel KH, Rubin AL: Interleukin-6 predicts hypoalbuminemia, hypocholesterolemia, and mortality in hemodialysis patients. *Am J Kidney Dis* 32: 107–114, 1998
8. Rao M, Guo D, Perianayagam MC, Tighiouart H, Jaber BL, Pereira BJ, Balakrishnan VS: Plasma interleukin-6 predicts cardiovascular mortality in hemodialysis patients. *Am J Kidney Dis* 45: 324–333, 2005
9. Kaizu Y, Ohkawa S, Odamaki M, Ikegaya N, Hibi I, Miyaji K, Kumagai H: Association between inflammatory mediators and muscle mass in long-term hemodialysis patients. *Am J Kidney Dis* 42: 295–302, 2003
10. Honda H, Qureshi AR, Heimbürger O, Barany P, Wang K, Pecoits-Filho R, Stenvinkel P, Lindholm B: Serum albumin, C-reactive protein, interleukin 6, and fetuin a as predictors of malnutrition, cardiovascular disease, and mortality in patients with ESRD. *Am J Kidney Dis* 47: 139–148, 2006
11. Barreto DV, Barreto FC, Liabeuf S, Temmar M, Lemke HD, Tribouilloy C, Choukroun G, Vanholder R, Massy ZA; European Uremic Toxin Work Group (EUTox): Plasma interleukin-6 is independently associated with mortality in both hemodialysis and pre-dialysis patients with chronic kidney disease. *Kidney Int* 77: 550–556, 2010
12. Kojima M, Hosoda H, Date Y, Nakazato M, Matsuo H, Kangawa K: Ghrelin is a growth-hormone-releasing acylated peptide from stomach. *Nature* 402: 656–660, 1999
13. Kojima M, Kangawa K: Ghrelin: Structure and function. *Physiol Rev* 85: 495–522, 2005
14. Date Y, Kojima M, Hosoda H, Sawaguchi A, Mondal MS, Suganuma T, Matsukura S, Kangawa K, Nakazato M: Ghrelin, a novel growth hormone-releasing acylated peptide, is synthesized in a distinct endocrine cell type in the gastrointestinal tracts of rats and humans. *Endocrinology* 141: 4255–4261, 2000
15. Pérez-Fontán M, Cordido F, Rodríguez-Carmona A, Peteiro J, García-Naveiro R, García-Buela J: Plasma ghrelin levels in patients undergoing haemodialysis and peritoneal dialysis. *Nephrol Dial Transplant* 19: 2095–2100, 2004
16. Yoshimoto A, Mori K, Sugawara A, Mukoyama M, Yahata K, Suganami T, Takaya K, Hosoda H, Kojima M, Kangawa K, Nakao K: Plasma ghrelin and desacyl ghrelin concentrations in renal failure. *J Am Soc Nephrol* 13: 2748–2752, 2002
17. Aguilera A, Cirugeda A, Amair R, Sansone G, Alegre L, Codoceo R, Bajo MA, del Peso G, Díez JJ, Sánchez-Tomero JA, Selgas R: Ghrelin plasma levels and appetite in peritoneal dialysis patients. *Adv Perit Dial* 20: 194–199, 2004
18. Jarkovská Z, Hodková M, Sazamová M, Rosická M, Dusilová-Sulková S, Marek J, Justová V, Lacinová Z, Haluzík M, Haas T, Krsek M: Plasma levels of active and total ghrelin in renal failure: A relationship with GH/IGF-I axis. *Growth Horm IGF Res* 15: 369–376, 2005
19. Jarkovská Z, Rosická M, Krsek M, Sulková S, Haluzík M, Justová V, Lacinová Z, Marek J: Plasma ghrelin levels in patients with end-stage renal disease. *Physiol Res* 54: 403–408, 2005
20. Iglesias P, Díez JJ, Fernández-Reyes MJ, Codoceo R, Alvarez-Fidalgo P, Bajo MA, Aguilera A, Selgas R: Serum ghrelin concentrations in patients with chronic renal failure undergoing dialysis. *Clin Endocrinol (Oxf)* 64: 68–73, 2006
21. Rodriguez Ayala E, Pecoits-Filho R, Heimbürger O, Lindholm B, Nordfors L, Stenvinkel P: Associations between plasma ghrelin levels and body composition in end-stage renal disease: A longitudinal study. *Nephrol Dial Transplant* 19: 421–426, 2004
22. Liu J, Prudom CE, Nass R, Pezzoli SS, Oliveri MC, Johnson ML, Veldhuis P, Gordon DA, Howard AD, Witcher DR, Geysen HM, Gaylinn BD, Thorner MO: Novel ghrelin assays provide evidence for independent regulation of ghrelin acylation and secretion in healthy young men. *J Clin Endocrinol Metab* 93: 1980–1987, 2008
23. Prudom C, Liu J, Patrie J, Gaylinn BD, Foster-Schubert KE, Cummings DE, Thorner MO, Geysen HM: Comparison of competitive radioimmunoassays and two-site sandwich assays for the measurement and interpretation of plasma ghrelin levels. *J Clin Endocrinol Metab* 95: 2351–2358, 2010
24. Pérez-Fontán M, Cordido F, Rodríguez-Carmona A, García-Naveiro R, Isidro ML, Villaverde P, García-Buela J: Acute plasma ghrelin and leptin responses to oral feeding or intraperitoneal hypertonic glucose-based dialysate in patients with chronic renal failure. *Kidney Int* 68: 2877–2885, 2005
25. 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
26. Ozkaya O, Buyan N, Bideci A, Gonen S, Ortac E, Fidan K, Cinaz P, Söylemezoğlu O: Osteoprotegerin and RANKL serum levels and their relationship with serum ghrelin in children with chronic renal failure and on dialysis. *Nephron Clin Pract* 105: c153–c158, 2007
27. Beaumont NJ, Skinner VO, Tan TM, Ramesh BS, Byrne DJ, MacColl GS, Keen JN, Bouloux PM, Mikhailidis DP, Bruckdorfer KR, Vanderpump MP, Srai KS: Ghrelin can bind to a species of high density lipoprotein associated with paraoxonase. *J Biol Chem* 278: 8877–8880, 2003
28. Patterson M, Murphy KG, le Roux CW, Ghatei MA, Bloom SR: Characterization of ghrelin-like immunoreactivity in human plasma. *J Clin Endocrinol Metab* 90: 2205–2211, 2005
29. Akamizu T, Shinomiya T, Irako T, Fukunaga M, Nakai Y, Nakai Y, Kangawa K: Separate measurement of plasma levels of acylated and desacyl ghrelin in healthy subjects using a new direct ELISA assay. *J Clin Endocrinol Metab* 90: 6–9, 2005
30. Lowrie EG, Lew NL: Death risk in hemodialysis patients: The predictive value of commonly measured variables and an evaluation of death rate differences between facilities. *Am J Kidney Dis* 15: 458–482, 1990
31. Stenvinkel P, Heimbürger O, Paultre F, Diczfalusy U, Wang T, Berglund L, Jogestrand T: Strong association between malnutrition, inflammation, and atherosclerosis in chronic renal failure. *Kidney Int* 55: 1899–1911, 1999
32. Levey AS, Bosch JP, Lewis JB, Greene T, Rogers N, Roth D; Modification of Diet in Renal Disease Study Group: A more accurate method to estimate glomerular filtration rate from serum creatinine: A new prediction equation. *Ann Intern Med* 130: 461–470, 1999
33. Deboer MD, Zhu X, Levasseur PR, Inui A, Hu Z, Han G, Mitch WE, Taylor JE, Halem HA, Dong JZ, Datta R, Culler MD, Marks DL: Ghrelin treatment of chronic kidney disease: improvements in lean body mass and cytokine profile. *Endocrinology* 149: 827–835, 2008
34. Barazzoni R, Zhu X, Deboer M, Datta R, Culler MD, Zanetti M, Guarnieri G, Marks DL: Combined effects of ghrelin and higher food intake enhance skeletal muscle mitochondrial oxidative capacity and AKT phosphorylation in rats with chronic kidney disease. *Kidney Int* 77: 23–28, 2010
35. Wynne K, Giannitsopoulou K, Small CJ, Patterson M, Frost G, Ghatei MA, Brown EA, Bloom SR, Choi P: Subcutaneous ghrelin

enhances acute food intake in malnourished patients who receive maintenance peritoneal dialysis: A randomized, placebo-controlled trial. *J Am Soc Nephrol* 16: 2111–2118, 2005
36. Ashby DR, Ford HE, Wynne KJ, Wren AM, Murphy KG, Busbridge M, Brown EA, Taube DHGMA, Ghatei MA, Tam FW, Bloom SR, Choi P: Sustained appetite improvement in malnourished dialysis patients by daily ghrelin treatment. *Kidney Int* 76: 199–206, 2009
37. Muscaritoli M, Molfino A, Chiappini MG, Laviano A, Ammann T, Spinsanti P, Melchiorri D, Inui A, Alegiani F, Rossi Fanelli F: Anorexia in hemodialysis patients: The possible role of des-acyl ghrelin. *Am J Nephrol* 27: 360–365, 2007
38. Kirk SE, Gertz BJ, Schneider SH, Hartman ML, Pezzoli SS, Wittreich JM, Krupa DA, Seibold JR, Thorner MO: Effect of obesity and feeding on the growth hormone (GH) response to the GH secretagogue L-692,429 in young men [Abstract. *J Clin Endocrinol Metab* 82: 1154–1159, 1997
39. Chapman IM, Pescovitz OH, Murphy G, Treep T, Cerchio KA, Krupa D, Gertz B, Polvino WJ, Skiles EH, Pezzoli SS, Thorner MO: Oral administration of growth hormone (GH) releasing peptide-mimetic MK-677 stimulates the GH/insulin-like growth factor-I axis in selected GH-deficient adults. *J Clin Endocrinol Metab* 82: 3455–3463, 1997
40. Murphy MG, Bach MA, Plotkin D, Bolognese J, Ng J, Krupa D, Cerchio K, Gertz BJ: Oral administration of the growth hormone secretagogue MK-677 increases markers of bone turnover in healthy and functionally impaired elderly adults. The MK-677 Study Group. *J Bone Miner Res* 14: 1182–1188, 1999
41. Murphy MG, Plunkett LM, Gertz BJ, He W, Wittreich J, Polvino WM, Clemmons DR: MK-677, an orally active growth hormone secretagogue, reverses diet-induced catabolism. *J Clin Endocrinol Metab* 83: 320–325, 1998
42. Bresciani E, Nass R, Torsello A, Gaylinn B, Avallone R, Locatelli V, Thorner MO, Müller EE: Hexarelin modulates the expression of growth hormone secretagogue receptor type 1a mRNA at hypothalamic and pituitary sites. *Neuroendocrinology* 80: 52–59, 2004
43. Iglesias P, Díez JJ, Fernández-Reyes MJ, Aguilera A, Burgués S, Martínez-Ara J, Miguel JL, Gómez-Pan A, Selgas R: Recombinant human growth hormone therapy in malnourished dialysis patients: A randomized controlled study. *Am J Kidney Dis* 32: 454–463, 1998
44. Asakawa A, Inui A, Fujimiya M, Sakamaki R, Shinfuku N, Ueta Y, Meguid MM, Kasuga M: Stomach regulates energy balance via acylated ghrelin and desacyl ghrelin. *Gut* 54: 18–24, 2005
45. Chen C-Y, Inui A, Asakawa A, Fujino K, Kato I, Chen C-C, Ueno N, Fujimiya M: Des-acyl ghrelin acts by CRF type 2 receptors to disrupt fasted stomach motility in conscious rats. *Gastroenterology* 129: 8–25, 2005
46. Arbeiter AK, Büscher R, Petersenn S, Hauffa BP, Mann K, Hoyer PF: Ghrelin and other appetite-regulating hormones in paediatric patients with chronic renal failure during dialysis and following kidney transplantation. *Nephrol Dial Transplant* 24: 643–646, 2009
47. Bossola M, Muscaritoli M, Valenza V, Panocchia N, Tazza L, Cascino A, Laviano A, Liberatori M, Lodovica Moussier M, Rossi Fanelli F, Luciani G: Anorexia and serum leptin levels in hemodialysis patients. *Nephron Clin Pract* 97: c76–c82, 2004
48. Dixit VD, Schaffer EM, Pyle RS, Collins GD, Sakthivel SK, Palaniappan R, Lillard JW Jr, Taub DD: Ghrelin inhibits leptin- and activation-induced proinflammatory cytokine expression by human monocytes and T cells. *J Clin Invest* 114: 57–66, 2004
49. Pérez-Fontán M, Cordido F, Rodríguez-Carmona A, Penín M, Díaz-Cambre H, López-Muñiz A, Sangiao-Alvarellos S, García-Buela J: Short-term regulation of peptide YY secretion by a mixed meal or peritoneal glucose-based dialysate in patients with chronic renal failure. *Nephrol Dial Transplant* 23: 3696–3703, 2008

Received: September 6, 2012 **Accepted:** January 29, 2013

Published online ahead of print. Publication date available at www.cjasn.org.

Present address: Dr. Rohit Gupta, Nephrology Associates of Chattanooga, Chattanooga, Tennessee; Dr. Tamil Kuppusamy, Mid-Atlantic Nephrology Associates, P.A., Catonsville, Maryland.

This article contains supplemental material online at http://cjasn.asnjournals.org/lookup/suppl/doi:10.2215/CJN.09170912/-/DCSupplemental.

SUPPLEMENTAL MATERIAL

Study Protocol Details:

Subjects were enrolled from the University of Virginia Kidney Center Clinic and dialysis unit. All patients in clinic were being seen for kidney related diseases. Qualifying laboratory data were based on values from the patients clinic visit immediately prior to the study visit, . All were referred for renal disease including hypertension, diabetes, stones, IgA nephropathy, Sjögrens, polycystic kidney disease, vasculitis, membranous nephropathy, focal sclerosis, lithium induced nephropathy, reflux nephropathy and Henoch Schoenlein purpura. Twenty patients had diabetes, 10 of whom took insulin, most had proteinuria and many had diabetes with hypertension. All had evidence of renal disease. Our hospital reports an estimated glomerular filtration rate (GFR) for all serum using the Modification of Diet in Renal Disease (MDRD) formula. We screened our Kidney Center Clinic patients for eligibility and requested their participation in the study into the 5 stages of CKD as defined by the Kidney Disease Outcomes Quality Initiative (KDOQI) Clinical Practice Guidelines. They were not selected for any reason other than their CKD class, willingness to participate, and eligibility to achieve 10 subjects per group. Hemodialysis patents were recruited based on their monthly laboratory studies. Screening information was obtained from the monthly laboratory measurements immediately prior to the study visit. Four patients had diabetes as the cause of their ESRD and two of these took insulin. Seven had hypertension, one each had PKD, HIVAN, glomerulonephritis, and chronic interstitial nephritis. Criteria for exclusion included pregnancy, previous gastric surgery and recent use of prednisone, amphetamines, appetite stimulants or depressants within the past 2 weeks. Following the informed consent process, participants underwent a physical

examination and were asked questions about their medical history, review of systems, and current medications.

Assays for Hormones and Cytokines:

The following assays were performed in duplicate in the General Clinical Research Center (GCRC) core laboratory. GH and IGF -1 levels were determined using the Nichols Advantage automated chemiluminescence which uses acridinium ester chemiluminescence (Nichols Institute Diagnostics, San Clemente, CA. Insulin and hsCRP were quantitated on the Diagnostic Products Corporation Immulite 2000 Automated Immunoassay Analyzer using antibody-coated beads followed by alkaline phosphate reagents, (Los Angeles, CA). Both IL-6 and TNF alpha were determined by the quantitative sandwich enzyme immunoassay from R & D Systems (Minneapolis, MN). Leptin was assayed by an enzyme-linked immunosorbent assay from Diagnostic Systems Laboratory (Webster, TX). The intra-assay and inter-assay coefficients of variation for these assays are listed below.

	Intra-Assay	Inter-assay
GH	2.1%	3.4%
IGF-1	3.4%	5.9%
Insulin	2.2%	4.8%
hsCRP	3.1%	4.8%
IL-6	3.6%	8.6%
TNF-A	6.0%	12.1%
Leptin	2.4%	8.2%

Other Tables:

Tables 1S-5S contain the details of data analyses described in the text of the manuscript

Table 1S. Appetite classification

CKD Stage	Poor	Fair	Good
1	1 (11.1)	1 (11.1)	7 (77.8)
2	1 (10.0)	1 (10.0)	8 (80.0)
3	1 (9.1)	2 (18.2)	8 (72.7)
4	0 (0)	2 (20.0)	8 (80.0)
5	1 (10.0)	1 (10.0)	7 (70.0)

Pearson chi-square exact test for comparing appetite classification between CKD stages; P = 1.00

Table 2S. Meal duration (mins)

CKD Stage	n	Mean	SD	Median	25th Percentile	75th Percentile	Min	Max
1	9	12.9	3.8	12.0	10.0	14.0	10.0	20.0
2	10	13.9	4.6	13.0	10.3	17.8	7.0	20.0
3	11	12.0	5.7	14.0	10.5	15.0	0.0	20.0
4	10	13.5	4.6	13.5	11.0	17.3	5.0	20.0
5	10	13.2	5.3	10.0	10.0	14.3	10.0	25.0

Kruskal Wallis Test for no difference between the median meal duration; P=0.88

Table 3S. Percentage of breakfast intake.

CKD - Stage	n	Mean	SD	Median	25th Percentile	75th Percentile	Min	Max
1	9	94.4	11.0	100.0	100.0	100.0	75.0	100.0
2	10	87.5	24.3	100.0	81.3	100.0	25.0	100.0
3	11	86.4	17.2	100.0	75.0	100.0	50.0	100.0
4	10	85.0	17.5	87.5	75.0	100.0	50.0	100.0
5	10	87.5	21.2	100.0	81.3	100.0	50.0	100.0

Kruskal Wallis Test for no difference between the median % breakfast intake; P=0.74.

Table4S. Multivariate Spearman Correlations

Dependent Variable →	Proportion of Preprandial Ghrelin in the Des-acyl Ghrelin Form		Proportion of Postprandial Ghrelin in the Des-acyl Ghrelin Form	
Partial Correlates ↓	Spearman-Partial-Correlation r_{sp} [95% CI]	Pvalue	Spearman Partial Correlation r_p [95% CI]	P-value
Age	0.21 [-0.12, 0.53]	0.17	0.18 [-0.12, 0.47]	0.26
IL6	0.17 [-0.13, 0.46]	0.27	0.21 [-0.06, 0.49]	0.19
Leptin	0.03 [-0.28, 0.33]	0.84	0.05 [-0.27, 0.35]	0.73
TNFa	-0.00 [-0.28, 0.34]	1.00	0.13 [-0.18, 0.42]	0.40
Insulin	0.04 [-0.29, 0.39]	0.80	0.09 [-0.25, 0.45]	0.55

Rank-partial-correlations (r_{sp}) for the identifying unique bivariate relationships between the proportion of ghrelin in the des-acyl form and age, interleukin 6, (IL6), leptin, tumor necrosis factor α, (TNFα), and insulin.

Table 5S: Univariate Spearman Rank Correlation Analyses

Correlate X	Correlate Y	Spearman Rank Correlation (r_s	95% Confidence Interval	P-value
Preprandial AG	Insulin	-0.57	[-0.84, -0.07]	0.02
Preprandial DG:	Insulin	-0.67	[-0.89, -0.23]	0.006
	Albumin	-0.64	[-0.87, -0.17]	0.01
Preprandial DG+AG	Albumin	-0.56	[-0.84, -0.05]	0.03
Postprandial AG:	IGF-1	-0.57	[-0.84. -0.06]	0.03
	Insulin	-0.51	[-0.82, -0.01]	0.05
Postprandial DG:	IGF-1	-0.60	[-0.86, -0.12]	0.02
	Insulin	-0.83	[-0.94, -0.53]	<0.001
	Leptin	-0.61	[-0.86, -0.13]	0.01
	Albumin	-0.59	[-0.85, -0.09]	0.02
Post-Dialysis DG:	GH	0.75	[0.37, 0.91]	<0.001
	CRP	0.59	[0.10, 0.85]	0.02
Change in AG				
Postprandial:	CRP	0.56	[0.06, 0.84]	0.03
	IL6	0.54	[0.03, 0.83]	0.04
Post Dialysis – Postprandial:	IGF-1	0.53	[0.01, 0.83]	0.04
	TNFα	-0.74	[-0.91, -0.35]	0.002
	IL6	-0.52	[-0.82, 0.01]	0.05
Change in DG:				
Postprandial	Insulin	0.54	[0.02, 0.83]	0.04
Change in DG+AG Post Dialysis	CRP	0.63	[0.16, 0.87]	0.01
GH:	IGF-1	-0.65	[-0.88, -0.19]	0.009
	Insulin	-0.71	[-0.90. -0.30]	0.003
	Leptin	-0.67	[-0.88, -0.23]	0.006
IGF-1:	Insulin	0.79	[0.46, 0.93]	<0.001
	Leptin	0.59	[0.01, 0.85]	0.02
	Albumin	0.65	[0.19, 0.88]	0.008
Insulin:	Leptin	0.81	[0.50, 0.94]	<0.001
	Albumin	0.59	[0.10, 0.85]	0.02
CRP	IL6	0.53	[0.00, 0.82]	0.04

Correlations ($r_{s)}$ for identifying bivariate relationships between the ghrelin, insulin, albumin, insulin like growth hormone-1, (IGF-1), growth hormone, (GH), C-reactive protein, (CRP), interleukin 6, (IL6), tumor necrosis factor α, (TNFα) measurements of the hemodialysis patients.