

Is there association between acyl-ghrelin and inflammation in hemodialysis patients?

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ABSTRACT

Introduction and Objectives: Patients with chronic kidney disease (CKD) present anorexia, which may be related with the chronic inflammatory process. Thus the objective of this study was to evaluate if there is association between inflammation and the orexigenic hormone, acyl-ghrelin, in CKD patients undergoing hemodialysis (HD).

Methods: Thirty-six patients were studied (61.1% men, 46.7 ± 14.9 years, BMI 22.9 ± 3.9 kg/m²) in regular HD program (65.0 ± 46.8 months). Plasma levels of acyl-ghrelin and inflammatory markers TNF- α , IL-6 and CRP were measured by enzyme immunoassay (ELISA, Enzyme Linked Immunosorbent Assay). Anthropometric parameters were collected for assessment of nutritional status and dietary intake was assessed by food recall. **Results:** The patients presented elevated plasma levels of IL-6 (83 ± 10 pg/mL), TNF- α (21.06 pg/mL [20.6-40.0]) and CRP (2.7 pg/mL [1.7-3.4]) compared to normal values. Acyl-ghrelin plasma levels were (18.0 [1.3 to 77.7 pg/mL]) low when compared to healthy individuals. However, patients with high BMI (> 25 kg/m²) presented lower acyl-ghrelin plasma levels (13.6 [1.3 to 30.5] pg/mL) when compared to patients with BMI < 25 kg/m² (21.7 [7.4 to 77.7] pg/mL) ($p < 0.05$). Acyl-ghrelin and BMI were negatively correlated ($r = -0.38$, $p = 0.02$) and there was no significant correlation between acyl-ghrelin and inflammatory markers.

Conclusions: Hemodialysis patients showed low acyl-ghrelin levels and seem to present an acyl-ghrelin resistance and there was no correlation between inflammation and this orexigenic hormone.

Keywords: dialysis; ghrelin; inflammation; renal insufficiency, chronic.

INTRODUCTION

Patients with chronic kidney disease (CKD) experience persisting inflammation and a series of ensuing complications. Cytokines such as interleukin-6 (IL-6) and tumor necrosis factors (TNF- α) act directly upon the central nervous system to introduce alterations in neurotransmitter release and function, leading to disordered appetite and dysfunctional energy metabolism¹⁻⁵ and the onset of wasting syndrome, consisting of anorexia, increased energy expenditure, reduction of protein stocks, and loss of fat and muscle tissue. Each individual component in this syndrome adds to the risk of death of patients with CKD.⁶ Inflammatory processes in CKD patients seem to be related to anorexia, which by its turn is a direct contributor to higher hospitalization rates, lower quality of life, and higher death rates in hemodialysis (HD) patients.⁷ Indeed, Zabel *et al.*⁸ found a positive correlation between appetite reductions and inflammatory markers in HD patients. Other authors have also verified correlations between high levels of pro-inflammatory cytokines and some appetite-regulating hormones.⁹⁻¹¹

One of the many hormones studied in CKD is ghrelin, a peptide with 28 amino acids secreted by the stomach which, when released, stimulates the appetite through the central nervous system (CNS) and leads to weight and body fat gain.¹² Two forms of ghrelin are found in bodily tissues and fluids: acyl-ghrelin, the peptide's active form, and des-acyl-ghrelin, which accounts for 80%-90% of the circulating

ghrelin. Des-acyl-ghrelin appears to act as an anorexigen and its levels are significantly increased in CKD patients as a consequence of poor renal clearance.^{13,14} Higher ghrelin levels may represent an attempt to increase the appetite and the anabolic activity of the growth hormone. However, in CKD patients the hormonal axis may be negatively regulated because of inflammation and uremia.¹⁵

Administration of ghrelin to rats with CKD improved food intake, muscle mass, and reduced muscle protein degradation and levels of inflammatory cytokines.¹⁶ In fact, ghrelin seems to be a potent anti-inflammatory agent in the immune system and in human endothelial cells, where it probably inhibits the expression of inflammatory cytokines IL-1 β , IL-6, and TNF- α .¹⁷

Nonetheless, recent studies have indicated that the levels of total ghrelin and acyl-ghrelin are altered in inflammatory conditions.⁹⁻¹¹ Total ghrelin and acyl-ghrelin levels seem to be negatively correlated to inflammatory cytokines in patients with chronic kidney disease undergoing hemodialysis, particularly TNF- α and IL-6.¹¹

Conflicting results have been published about this association. Therefore, this study aimed to analyze the correlation between acyl-ghrelin and inflammatory cytokines (IL-6, TNF- α , and CRP) in CKD patients undergoing hemodialysis.

MATERIALS AND METHODS

THE SERIES

This cross-sectional study included 36 patients (61.2% males) with CKD from the Renalvida Clinic (Rio de Janeiro, Brazil). The study enrolled patients meeting the following criteria: men and women aged between 18 and 65 years on hemodialysis for over three months with arteriovenous fistulas as the access method. Patients with inflammatory, acute or malignant disease were excluded. The mean duration of HD sessions was of approximately three to four and a half hours. Subjects underwent HD three times a week, with blood flow rates above 250 mL/min, dialysate flow rates of 500 mL/min, and bicarbonate buffer. The main causes for CKD were hypertensive nephrosclerosis (N = 24), followed by chronic glomerulonephritis (N = 6), diabetic nephrosclerosis (N = 2), polycystic kidney disease (N = 1) and other diseases or unknown causes (N = 3). This

study was approved by the Ethics Committee of the medical School of the Fluminense Federal University (073/10). All patients gave informed consent for their participation in the study.

NUTRITIONAL ASSESSMENT

The following anthropometric parameters were measured: circumference of the arm and waist, dry weight, and height for the calculation of the BMI. Tricipital, subscapular, suprailiac, and biceps skin fold measurements were made to calculate body fat percentages and body density by the summation of the four skin folds, according to the procedure described by Durnin & Womersley,¹⁸ and body fat percentages as per Siri's¹⁹ equation. Measurements were made after hemodialysis sessions by a trained professional.

Nutritional status was assessed based on the BMI (kg/m²), calculated by the ratio between dry weight and height to the square and categorized in accordance with the recommendations of the World Health Organization.²⁰ The cutoff points proposed by Lohman *et al.*²¹ were used as reference values for body fat percentages.

Corrected arm muscle area (CAMA) was used to improve the assessment of muscle tissue reserves, due to the correction for bone area. CAMA-based nutritional classification was carried out as per the procedures and reference values published by Frisancho.²² Waist circumference measurements were made to analyze the profile of body fat distribution of the patients. Measured values were compared to borderline values associated with risk of complications related to obesity. Increased risk was seen when waist circumference was greater than 102 cm for males and 88 cm for females.²³

Mean daily caloric and protein intake was estimated using 24-hour recall two-day food records (one day on hemodialysis and another off). Mean daily nutrient intake was calculated using the NutWin software package (developed by the Nutrition Department of the Federal University of São Paulo - UNIFESP, São Paulo, Brazil).

BIOCHEMICAL VARIABLES

Routine biochemical tests were collected from patient charts and categorized in accordance with the reference values described by the National Kidney

Foundation (NKF, 2000).²⁴ Dialysis dosage (Kt/V) was calculated from blood urea values before and after dialysis, weight, and duration of hemodialysis as described by Daugirdas.²⁵ Fast blood samples were taken before hemodialysis sessions. Samples were centrifuged (15 min, 3000 x g, 4°C) and plasma was stored at -80°C for future analysis. Plasma levels of acyl-ghrelin were analyzed using a commercially available kit (Human acylated Ghrelin EIA - SPI Bio, Montigny, France) and the values were compared to acyl-ghrelin levels of 18 healthy individuals (51 ± 6.6 years; seven males, 11 females). Pro-inflammatory cytokines were analyzed through immunoenzyme assays (human C-reactive protein, DY1707, R&D Systems®; human TNF- α /TNFSF1A, DY210, R&D Systems®; human IL-6, DY206, R&D Systems®) and values compared to those of a group of healthy individuals included in a study developed by our group.²⁶

STATISTICAL ANALYSIS

Results were expressed in the form of mean values \pm SD (standard deviation) or minimum and maximum values as per the Shapiro-Wilk test of normality. Plasma acyl-ghrelin, TNF- α and CRP did not present normal distributions. The Mann-Whitney test was applied to analyze the differences between mean values. The *t*-test for independent samples was applied to analyze the differences between the mean values of the variables that followed a normal distribution. Variables were analyzed for gender and BMI (cutoff point set at 25.0 kg/m²). The correlation coefficients between the variables were calculated using Pearson's or Spearman's coefficient as needed. Statistical significance was assigned for events with *p* < 0.05. Statistical analysis was carried out using software package SPSS (Statistical Package for the Social Sciences) 17.0 (Chicago, EUA).

RESULTS

Table 1 shows anthropometric and biochemical parameters. Fourteen percent of the patients had BMIs consistent with malnutrition (< 18.5 kg/m²) and 44.4% were overweight or obese (BMI > 25 kg/m²). All patients had high percentages of body fat and about 20% of the male and 43% of the female individuals had waist circumference measurements above NCEP (2001)²³ recommendations. Contrastingly, 66.7% of the patients had nutrient depletion based on the corrected arm muscle area measurement.

TABLE 1 MEAN VALUES AND STANDARD DEVIATIONS FOR ANTHROPOMETRIC AND BIOCHEMICAL PARAMETERS

Parameter	Total (n = 36)	Males (n = 22)	Females (n = 14)
Age (years)	46.0 \pm 15.2	44.0 \pm 12.6	49.4 \pm 18.8
Time on HD (months)	65.0 \pm 46.8	60.0 \pm 49.2	72.8 \pm 42.5
Kt/V	1.5 \pm 0.4	1.4 \pm 0.3	1.7 \pm 0.3
BMI (kg/m ²)	22.9 \pm 3.9	23.2 \pm 4.1	22.3 \pm 3.7
CAMA (cm ²)	29.5 \pm 12.9	30.2 \pm 14.3	25.9 \pm 13.3
Fat %	30.4 \pm 6.8	27.4 \pm 5.9*	35.2 \pm 5.1
FFM (kg)	43.1 \pm 9.6	48.1 \pm 8.6*	35.2 \pm 4.7
WC (cm)	81.5 \pm 12.1	83.3 \pm 11.2	78.6 \pm 13.4
Albumin (g/dL)	3.6 \pm 0.2	3.6 \pm 0.3	3.6 \pm 0.2
Hematocrit (%)	32.7 \pm 4.8	32.7 \pm 4.8	32.8 \pm 4.9
Hemoglobin (g/dL)	10.8 \pm 1.6	10.8 \pm 1.7	10.9 \pm 1.7

* *p* < 0.001; BMI: Body mass index; CAMA: Corrected arm muscle area; FFM: Fat-free mass; WC: Waist circumference.

Most individuals (70%) had caloric intakes under 35 kcal/kg/day, and only 30% had proper levels of energy intake. Mean protein intake was 1.4 \pm 0.9 g/kg/day, but half of the patients had less than 1.2 g/kg/day of proteins.

The biochemical data presented in Table 2 indicates that patients had high plasma concentrations of IL-6 (83.0 \pm 10.2 pg/mL), TNF- α (21.06 [20.6-40.0] pg/mL) and CRP (2.7 [1.7-3.4] pg/mL), when compared to healthy subjects - IL-6 (2.7 \pm 0.3 pg/mL), TNF- α (2.3 \pm 1.2 pg/mL), CRP (0.59 \pm 0.07 pg/mL).²⁶ Patients had lower acyl-ghrelin concentrations (18.0 [1.3-77.7] pg/mL) than healthy subjects (24.2 [16.3-41.7] pg/mL) (*p* < 0.001).

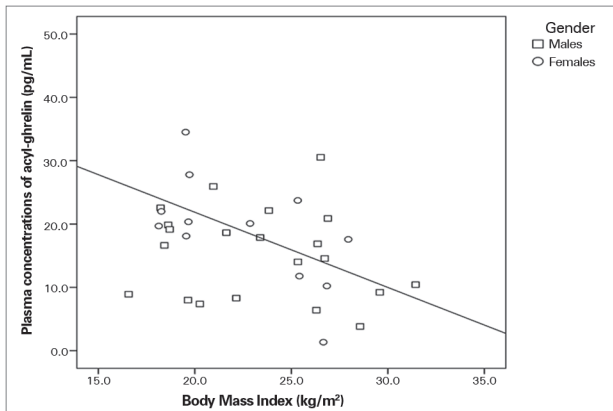
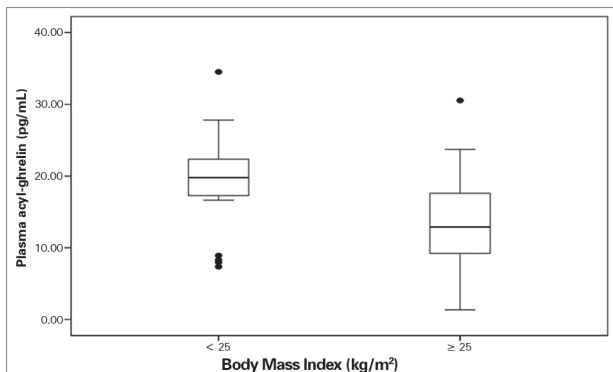
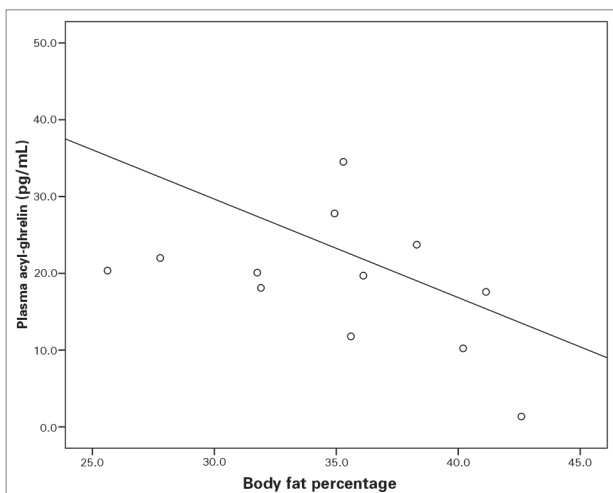
Plasma concentrations of acyl-ghrelin were negatively correlated with BMI (*r* = -0.38; *p* = 0.02), as seen in Figure 1. Overweight/obese patients had significantly lower levels of acyl-ghrelin (13.6 [1.3-30.5 pg/mL]) against individuals with a BMI under 25 kg/m² (21.7 [7.4-77.7] pg/mL), as indicated in Figure 2.

Among female subjects, body fat was negatively correlated with acyl-ghrelin (*r* = -0.53; *p* = 0.03) (Figure 3). There was no significant correlation between age, nutritional status, and pro-inflammatory cytokines, or between acyl-ghrelin concentrations and cytokines (TNF- α , IL-6, CRP).

TABLE 2 PLASMA CONCENTRATIONS OF ACYL-GHRELIN AND CYTOKINES IN HEMODIALYSIS PATIENTS

Biochemical parameters	Total (n = 36)	Males (n = 22)	Females (n = 14)
Acyl-ghrelin (pg/mL)	18.0 (1.3-77.7)	16.6 (3.83-30.54)	20.1 (1.35-77.7)
IL-6 (pg/mL)	83.0 ± 10.2	82.6 ± 9.7	83.7 ± 11.3
TNF-α (pg/mL)	21.1 (20.6-40.0)	21.1 (20.7-40.0)	21.0 (21.7-40.0)
CRP (pg/mL)	2.7 (1.7-3.4)	2.7 (2.4-3.3)	2.7 (1.7-3.3)

IL-6: Interleukin-6; TNF: Tumor necrosis factor; CRP: C-reactive protein.

Figure 1. Correlation between plasma concentrations of acyl-ghrelin and BMI.**Figure 2.** Box-plot of plasma concentrations of acyl-ghrelin according to BMI.**Figure 3.** Correlation between plasma concentrations of acyl-ghrelin and body fat percentages in female patients.

DISCUSSION

Altered levels of ghrelin, one of the peptides that affect appetite and nutritional status, have been described in patients with CKD.^{27,28} In this study, patients on hemodialysis had lower levels of acyl-ghrelin than healthy subjects; additionally, patients with BMIs > 25 kg/m² had significantly lower plasma concentrations of acyl-ghrelin than individuals with BMIs < 25 kg/m². No correlations were observed between inflammatory markers and acyl-ghrelin levels.

High levels of total ghrelin have been described in CKD patients.^{13,27,29,30} However, a great deal of these studies reported on plasma concentrations of total ghrelin, while this study looked into acyl-ghrelin, an orexigen, unlike des-acyl-ghrelin, an anorexigen that makes up for 90% of the total circulating ghrelin.

Authors have also described low levels of acyl-ghrelin in CKD patients. Oner-Iyidogan *et al.*¹¹ assessed 36 hemodialysis patients and found low plasma concentrations of acyl-ghrelin. Mafra *et al.*¹² also found low plasma levels of acyl-ghrelin in 125 patients on hemodialysis. According to these authors, patients with CKD have reduced plasma concentrations of acyl-ghrelin and high levels of des-acyl-ghrelin, being the high values of total ghrelin explained by the des-acyl component. In fact, Muscaritoli *et al.*⁹ found significantly higher levels of des-acyl-ghrelin in hemodialysis patients than in healthy subjects.

This study revealed a negative correlation between BMI and plasma levels of acyl-ghrelin; in women, a strong negative correlation was found between acyl-ghrelin and body percent fat. These findings agree with Mafra *et al.*,¹² according to whom acyl-ghrelin levels were higher in patients with a BMI < 23 kg/m². Likewise, when total ghrelin was considered, some authors have found an inverse relation between total ghrelin and BMI, and CKD patients with cachexia and high levels of total ghrelin. In view of these findings, this profile has been suggested as a mechanism to maintain energy balance, avoiding weight loss in patients^{31,32} in whom

acyl-ghrelin is responsible for the orexigen effects of total ghrelin.³³⁻³⁵ Additionally, a recent study carried out in humans found that the expression of activating enzyme ghrelin O-acyltransferase (GOAT) is altered by different body weight conditions, thus neutralizing ghrelin's adaptive alterations observed in these conditions and contributing to the development or maintenance of anorexia or obesity.³⁶

In fact, some authors have shown an inverse relation between BMI, body fat, and acyl-ghrelin levels.³⁴ According to Chen *et al.*,³⁵ reductions in body weight increase ghrelin concentrations, while increases in body weight reduce ghrelin concentrations. Thin patients appear to have some form of ghrelin resistance, for which reason they have a tendency to develop anorexia. This fact is consistent with our findings. Although 70% of the individuals had food intake levels lower than recommended levels, subjects with a BMI under 25 kg/m² had higher plasma concentrations of acyl-ghrelin than obese patients.

Lower levels of acyl-ghrelin may be correlated with inflammation in patients with high levels of cytokines. However, no significant correlations were seen between inflammation, energy-protein intake, and acyl-ghrelin levels in this study.

Pro-inflammatory cytokines lead to protein catabolism and muscle mass reduction.⁶ In this study, a large portion of the patients had muscle depletion, despite the lack of significant correlations between pro-inflammatory cytokines and muscle tissue. According to Carrero & Stenvinkel,³⁷ pro-inflammatory cytokines, particularly IL-6, play an important role in muscle catabolism and contribute to the onset of wasting, a condition seen in 23% to 76% of patients on hemodialysis characterized by caloric-protein depletion. This nutritional status deterioration is characterized by anorexia, high energy expenditure, low protein serum levels, loss of weight and muscle tissue.^{6,38,39}

Fat is known to be an endocrine tissue that produces and secretes various pro-inflammatory cytokines; one of them is IL-6, a key component in inflammatory processes. Apparently, high plasma concentrations of IL-6 are strongly correlated with the BMI in obese patients.⁴⁰ Additionally, according to Mafrá *et al.*,⁴¹ obesity may be a factor in subclinical inflammation, in which increases in fat tissue lead to increases in pro-inflammatory cytokines such as IL-6. Although the

patients in this study had high body fat percentages, no correlation was established between this finding and inflammatory cytokines.

There is conflicting information on the correlation between total ghrelin, acyl-ghrelin, and inflammatory markers. Studies have provided evidences showing that total ghrelin may have anti-inflammatory effects by reducing the expression of pro-inflammatory cytokines.^{17,28} According to Yada *et al.*,⁴² acyl-ghrelin appears to regulate the proliferation of immune cells, the activation and secretion of pro-inflammatory cytokines, thus inhibiting the expression of pro-inflammatory cytokines such as TNF- α and IL-6.⁴³

In sum, the plasma concentrations of acyl-ghrelin of patients on hemodialysis were found to be lower than in healthy subjects; these concentrations were negatively correlated with BMI and body fat percentage, thus establishing the clear impact of nutritional status on acyl-ghrelin levels. Additionally, the presence of inflammation was evident in view of the levels of pro-inflammatory cytokines mentioned above, despite the absence of correlations with acyl-ghrelin levels.

Considering the important properties of ghrelin and its derivatives in CKD and the many contradictory results published in the literature, more studies are required to clarify the correlation between acyl-ghrelin and inflammation in patients with chronic kidney disease.

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