Nutrition 26 (2010) 1100–1104

Contents lists available at ScienceDirect

Nutrition

journal homepage: www.nutritionjrnl.com

Applied nutritional investigation

Obestatin and ghrelin interplay in hemodialysis patients

Denise Mafra Ph.D. [a,*], Fitsum Guebre-Egziabher M.D. [b], Christine Cleaud R.D. [c], Walid Arkouche M.D. [c], Anne Mialon Pharm.D. [d], Jocelyne Drai Pharm.D. [d], D. Fouque M.D., Ph.D. [b]

[a] Clinical Nutrition Department, Nutrition Faculty, Federal University Fluminense, Niterói, Brazil
[b] Department of Nephrology, Hôpital E. Herriot and INSERM U870, Université Lyon 1, Lyon, France
[c] Association pour l'utilisation du Rein Artificiel, Lyon, France
[d] Department of Biochemistry, Hôpital Jules Courmont, Pierre Benite, France

ARTICLE INFO

Article history:
Received 25 March 2009
Accepted 1 September 2009

Keywords:
Ghrelin
Obestatin
Chronic kidney disease
Appetite
Nutrition
Hemodialysis

ABSTRACT

Objective: Compounds involved in the regulation of appetite and body composition appear to be of interest in chronic kidney disease. The purpose of this study was to analyze plasma obestatin and acyl and des-acyl ghrelin in patients on hemodialysis (HD).
Methods: Fifty patients on HD (56.0% women, mean age 62.2 ± 15.2 y) were studied. Blood samples were collected during fasting, before a regular HD session. Serum acyl and des-acyl ghrelin levels, leptin, and obestatin were measured using enzyme immunometric assay methods. Anthropometric parameters, appetite score, and food intake were recorded.
Results: Patients showed elevated serum leptin (34.1 ± 30 ng/mL), normal acyl ghrelin (137 ± 116.5 pg/mL), high des-acyl ghrelin (670 ± 479 pg/mL), and low obestatin (2.0 ± 1.4 ng/mL) levels compared with healthy volunteers. According to body mass index (BMI), patients with a BMI >23 kg/m^2 had significantly lower plasma obestatin. In contrast, leptin levels were increased and acyl ghrelin tended to be higher in these patients. There was a strong positive correlation between obestatin and des-acyl ghrelin ($r = 0.56$, $P = 0.0001$) and inverse correlations between obestatin and BMI ($r = -0.40$, $P = 0.007$), waist circumference ($r = -0.38$, $P = 0.024$), and C-reactive protein ($r = -0.29$, $P = 0.048$). By multivariate analysis, obestatin was independently and positively correlated with des-acyl ghrelin ($P = 0.01$), but not with C-reactive protein, BMI, or waist circumference.
Conclusion: In summary, patients on HD exhibited increased plasma levels of des-acyl ghrelin, normal acyl ghrelin levels, and low obestatin levels. In lean patients, the obestatin and des-acyl ghrelin levels were increased, suggesting that these hormones may influence appetite and body composition in patients on HD.

Introduction

Recent studies have led to the discovery of ghrelin, a stomach-derived circulating hormone that strongly stimulates food intake [1,2]. Ghrelin must be acylated at the serine-3 level to become active, and this acylated form of ghrelin (acyl ghrelin) is quite unstable and rapidly metabolized into the des-acylated form (des-acyl ghrelin) [3]. In addition to the ghrelin mature peptide, Zhang et al. [4] identified a 23–amino acid peptide with a flanking conserved glycine residue at the C-terminus. This compound was named *ghrelin-associated peptide*, or *obestatin*, and it has a negative effect on feeding. However, these anorexigenic properties of obestatin are still controversial [4–6]. It is interesting to note that these two peptides with potentially opposite actions on food intake and weight regulation are derived from the same ghrelin gene and released by the stomach [4].

Studies on ghrelin and obestatin levels in patients with chronic kidney disease (CKD) appear of interest because these compounds may be implicated in reduced appetite, loss of weight, and/or malnutrition. Studies have shown that plasma ghrelin is increased in patients on hemodialysis (HD), without any particular correlation with body composition, suggesting that there might be a resistance to ghrelin action in patients on HD [7–9]. However, virtually no study has independently

This work was supported by Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (Capes), Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) and Fundação Carlos Chagas Filho de Amparo d Pesquisa do Estado do Rio de Janeiro (FAPERJ).

* Corresponding author. Tel.: +55-21-2246-6022; fax: +55-21-2629-9847.
E-mail address: dmafra@terra.com.br (D. Mafra).

0899-9007/$ – see front matter © 2010 Elsevier Inc. All rights reserved.
doi:10.1016/j.nut.2009.09.003

examined acyl and des-acyl ghrelin levels in patients with CKD, and, at present, levels of obestatin in patients with CKD are unknown. The purpose of the present report was to describe the plasma obestatin and acyl and des-acyl ghrelin concentrations in patients on maintenance HD and their relations with appetite and body composition.

Materials and methods

Subjects

Fifty patients on maintenance HD (56.0% women, mean age 62.2 ± 15.2 y) were enrolled from the Hôpital Edouard Herriot and the Association pour l'utilisation du Rein Artificiel Clinic in Lyon, France. Inclusion criteria were an age older than 18 y and patients who had been on maintenance HD for at least 6 mo. Patients with inflammatory diseases or known malignancies were excluded. The dialysis sessions were 3–4.5 h three times per week, with a blood flow greater than 250 mL/min, a dialysate flow 500 mL/min, and bicarbonate buffer. Causes of renal failure were diabetes ($n = 20$), chronic glomerulonephritis ($n = 8$), nephroangiosclerosis ($n = 12$), and others ($n = 10$). The study protocol was approved by Le Comité de Protection des Personnes de Lyon, France. We also evaluated 50 healthy volunteers 32–58 y of age (66.0% women, mean age 44.2 ± 7.1 y), with a mean body mass index (BMI) of 23.8 ± 3.1 kg/m^2.

Methods

Nutritional assessment

The following anthropometric parameters were measured: body weight, height, waist circumference, and skinfold measurements at four standard sites (biceps, triceps, subscapular, and suprailiac) for determining body fat. Body density was calculated from the sum of the four skinfold measurements, according to Durnin and Womersley [10], and percentage of body fat was calculated by Siri's equation [11]. Measurements were made after the dialysis session by a trained staff member. Based on these measurements, the following indexes were calculated: BMI, arm muscle area, waist circumference, triceps skinfold, body fat percentage, and free-fat mass. BMI was calculated as weight divided by height squared. Triceps skinfold was measured with a Lange Skinfold Caliper (Cambridge Scientific Products, Cambridge, MA, USA), and the arm muscle area was calculated according to the following formula: ([midarm circumference {cm} − 3.14 × tricipital skinfold thickness {mm}]^2/4π) − n, where $n = 10$ for men and 6.5 for women [12]. Fat-free mass was calculated by subtracting fat mass from body weight. The corresponding percentiles were determined based on the tables developed by Frisancho [12], and values between percentiles 15 and 95 defined normality. The waist circumference values were based on the National Cholesterol Education Program's Adult Treatment Panel III [13].

The average daily intakes of calories, protein, carbohydrate, and fat were estimated using 2-d (dialysis day and non-dialysis day) food records. Average daily ingestion of nutrients was calculated using computerized diet software (Bilnut 4; S.C.D.A. NUTRISOFT, Cerelles, France). Appetite was rated by asking the following question: "How would you rate your appetite?" 1) very good, 2) good, 3) fair, 4) poor, or 5) very poor [14].

Biochemical variables

Serum albumin, prealbumin, glucose, C-reactive protein (CRP; by immunoturbidimetry, normal values <5 mg/L), parathormone (PTH), urea and creatinine were measured using standard laboratory methods.

Blood samples were obtained from the arterial line of the HD before the start of the session, after the patients had fasted overnight, and serum was immediately frozen at −20°C until analyzed. Serum acyl and des-acyl ghrelin levels were measured using the enzyme immunometric assay method (SPI Bio, Montigny, France). We calculated the ratios acyl ghrelin to obestatin and des-acyl to acyl ghrelin. Serum total leptin concentrations were measured by enzyme immunometric assay (SPI Bio). The reference value in healthy volunteers was 5.5 ± 4.0 ng/mL. We also analyzed these hormone levels according to BMI: group 1, a BMI lower than 23 kg/m^2 for patients with a low BMI (according to the International Society for Renal Nutrition and Metabolism [15]), and group 2, a BMI higher than 23 kg/m^2.

Statistical analysis

Results were expressed as mean ± standard deviation or percentage change, as needed. Student's t test was used to test the difference between means, and the Kruskal-Wallis test was used for non-parametric data. Spearman's correlation coefficient was searched to examine the relation between variables. Statistical significance was accepted as $P < 0.05$. Statistical analyses were performed with SPSS 16.0 (SPSS, Inc., Chicago, IL, USA).

Results

Clinical and biochemical characteristics of the subjects are listed in Table 1. Of the patients, 30% presented BMI values below 22.9 kg/m^2, 20% presented values from 23 to 25, and 50% above 25 kg/m^2. Body fat percentages ranged from 11.2% to 35.3% for men (mean 24.7 ± 6.2%) and from 16.0% to 42.8% for women (mean 33.0 ± 7.3%), and 88% had values above normal [16]. Of the patients, 28.0% and 15% presented triceps skinfold and arm muscle area values below the 15th percentile, and 55.6% of patients presented a large waist circumference. Daily protein intake was lower than 1.2 g/kg in 73.0% of patients, and mean was 1.03 ± 0.36 g · kg^{-1} · d^{-1}. Mean energy intake was 26.0 ± 9.3 kcal · kg^{-1} · d^{-1}; 82% had an energy intake lower than 35 kcal · kg^{-1} · d^{-1} (26–60 y old), and 72.7% lower than 30 kcal · kg^{-1} · d^{-1} (61–86 y old). Among 50 records, patient appetite scores were very good (9.8%), good (46.3%), satisfactory (29.3%), and poor (14.6%).

Serum albumin was 39.5 ± 2.5 g/L, and 24.0% patients had a serum albumin level lower than 38 g/L; 24.0% had a prealbumin level lower than 0.3 g/L (mean 0.35 ± 0.08 g/L). The mean CRP was 6.3 ± 8.6 mg/L, and 38% of patients presented a CRP above 5 mg/L. CRP was increased in patients with a BMI higher than 23 kg/m^2 (Table 1), but there was no significance. Leptin levels were above normal in 64.5% of patients, and des-acyl ghrelin levels were above 385 pg/mL in 74.5% of patients. The acyl ghrelin levels were below 63 pg/mL in 37.5% of patients, and 97% of the patients presented obestatin levels below 4.6 ng/dL. The des-acyl ghrelin moiety was strongly positively correlated with serum obestatin (Fig. 1; $r = 0.56$, $P < 0.0001$). According to BMI

Table 1
Patient characteristics and serum parameters according to BMI

	Mean ± SD (range) ($n = 50$)	BMI <23 kg/m^2 ($n = 15$)	BMI >23 kg/m^2 ($n = 35$)
Age (y)	62.0 ± 15.2 (26–86)	58.5 ± 19.2	63.4 ± 13.8
BMI (kg/m^2)	25.4 ± 4.4 (16.7–37.4)	20.6 ± 1.8^a	27.4 ± 3.4^b
Waist circumference (cm)	94.6 ± 14.0 (63–122.5)	81.6 ± 10.3^a	100.7 ± 11.4^b
Kt/V	1.5 ± 0.25 (0.9–2.0)	1.6 ± 0.2^a	1.45 ± 0.25^b
Albumin (g/L)	39.5 ± 2.5 (30.0–46.2)	39.3 ± 2.3	39.5 ± 2.7
Prealbumin (mg/dL)	0.35 ± 0.08 (0.12–0.57)	0.39 ± 0.07	0.34 ± 0.08
Glucose (mmol/L)	6.3 ± 2.7 (0.31–17)	4.2 ± 1.4^a	7.0 ± 2.8^b
CRP (mg/L)	6.3 ± 8.6 (0.2–43.6)	2.9 ± 2.7	7.2 ± 9.4
PTH (pg/mL)	404 ± 410.6 (2.5–2123)	449 ± 404	383.7 ± 404
Urea (mmol/L)	19.7 ± 4.7 (12–33.5)	18.6 ± 4.2	20.0 ± 4.9
Creatinine (μmol/L)	695 ± 135.6 (432–1026)	705.8 ± 118	695 ± 143

BMI, body mass index; CRP, C-reactive protein; Kt/V, K, dialyzer clearance of urea, t, dialysis time, V, patient's total body water; PTH, parathormone
Different superscript letters in the same line = $P < 0.05$.