Pediatric Nephrology (2018) 33:2113–2122
https://doi.org/10.1007/s00467-018-4002-7

ORIGINAL ARTICLE

Leptin and ghrelin in chronic kidney disease: their associations with protein-energy wasting

Nur Canpolat[1] · Lale Sever[1] · Ayse Agbas[1] · Mehmet Tasdemir[1] · Cigdem Oruc[1] · Ozlem Balcı Ekmekci[2] · Salim Caliskan[1]

Received: 19 February 2018 / Revised: 22 May 2018 / Accepted: 13 June 2018 / Published online: 6 July 2018

Abstract

Background This study aimed to evaluate plasma concentrations of leptin and total ghrelin in children with chronic kidney disease (CKD) and assess their roles in protein-energy wasting (PEW).

Methods This study consisted of three different CKD populations [CKD group (20 patients with non-dialysis CKD), dialysis group (39 patients on dialysis), and kidney transplant (KTx) group (35 KTx recipients)] and control group (18 healthy children). Plasma leptin and total ghrelin levels were measured. Multi-frequency bioimpedance analysis was used for the assessment of fat and lean mass. PEW was defined using criteria including body mass, muscle mass, growth, serum albumin level, and protein intake.

Results While plasma leptin levels did not differ among the study groups, total ghrelin levels were significantly higher in the dialysis group ($P < 0.001$). Seven dialysis patients (18%) and one CKD patient (5%) but none of the KTx recipients met the criteria of PEW. Dialysis patients with PEW had lower plasma leptin levels compared to their counterparts ($P = 0.018$); however, total ghrelin levels did not differ between the two groups ($P = 0.10$). Low leptin level in dialysis patients was independently associated with lower fat mass index ($P < 0.001$) and lower height-specific SD scores of BMI ($P = 0.019$).

Conclusions PEW is prevalent in dialysis patients. Low levels of leptin seem to be associated with PEW. Our result suggests that low leptin levels may be a consequence rather than a cause of PEW. Longitudinal studies are required to investigate this complex relationship between leptin and PEW in pediatric dialysis patients.

Keywords Chronic kidney disease · Dialysis · Ghrelin · Leptin · Protein-energy wasting · PEW · Children

Introduction

Protein-energy wasting (PEW) is a challenging state that affects many patients with chronic kidney disease (CKD), particularly those with end-stage kidney disease (ESKD). Inadequate protein and energy intake due to restricted diet and decreased appetite contribute to PEW in uremia [1, 2]. The mechanism underlying the decrease in appetite is not well understood. In addition to a uremic milieu and chronic inflammatory status, abnormalities of appetite regulating hormones and their complex interactions have been suggested as possible causative factors [3].

Leptin and ghrelin are two important hormones that play major roles in the regulation of food intake and also influence energy metabolism. Although there has been a great deal of interest in the roles of these two hormones in wasting syndromes associated with CKD, conflicting data have been reported. Leptin is an anorexigenic hormone, which induces weight loss by inhibiting appetite and increasing metabolic rate [4]. Hyperleptinemia has been suggested to cause PEW in uremic patients [5]; however, the contribution of leptin to the development of PEW is not well understood in this patient population. Some studies have identified hyperleptinemia in patients with CKD

This study was presented as a poster in the 50th annual meeting of the European Society for Pediatric Nephrology 2017.

✉ Nur Canpolat
nur.canpolat@istanbul.edu.tr

[1] Department of Pediatric Nephrology, Istanbul University Cerrahpasa Faculty of Medicine, 34098 Fatih, Istanbul, Turkey

[2] Department of Biochemistry, Istanbul University Cerrahpasa Faculty of Medicine, Istanbul, Turkey

and ESKD [6–10], while others reported no alterations in plasma leptin levels in these populations [11–14].

Ghrelin is another hormone that has been suggested to play a role in the regulation of appetite and maintenance of nutritional status. It is traditionally considered to be an appetite-stimulating hormone. However, this traditional definition of ghrelin has recently been debated and it seems to play a more complex role in the regulation of appetite and feeding behavior than simply stimulating food intake [15]. The metabolism of ghrelin is altered by uremia [16], and abnormalities in circulating levels of ghrelin in uremia have been linked to poor nutritional status and PEW [13, 17, 18].

Although diagnostic criteria for wasting syndromes have been well defined in adult CKD patients [19, 20], until recently, there have been no such diagnostic criteria for use in pediatric patients with CKD. The criteria for PEW have just been described in this population [21]. The present study was performed to evaluate plasma concentrations of leptin and ghrelin in relation to body composition in children with CKD as well as to assess their roles in PEW according to the recently described criteria.

Patients and methods

Study population

This single-center study included 94 patients with CKD (35 female) aged between 5 and 20 years, followed up between the years 2013 and 2015 at the Division of Pediatric Nephrology at Istanbul University Cerrahpasa Faculty of Medicine.

The study consisted of three different CKD populations. The first group comprised 20 patients with CKD stages II–IV (*CKD group*) with mean eGFR 52 ± 23 mL/min per 1.73 m^2. There were 39 patients on chronic dialysis (*Dialysis group*) in the second group. The Dialysis group included 22 peritoneal dialysis (PD) and 17 hemodialysis (HD) patients, with median dialysis duration of 18 (range 3-159) months. Eighteen PD patients were treated by automated PD, and the mean weekly kt/V of PD patients was 2.1 ± 0.5. All HD patients underwent a conventional low-flux standard HD, except two who were treated by high-flux HD, for 4–5 h/session thrice-weekly, and the median single pool kt/V was 1.8 ± 0.6. The third group consisted of 35 kidney transplant (KTx) recipients (*KTx group*) with a well-functioning graft [mean eGFR 71 ± 24 mL/min per 1.73 m^2 and median time since transplant of 35 (range 3–159) months]. The *Control group* consisted of 18 healthy children who were referred to pediatric outpatient clinics and had blood samples taken for any reason. Children with known chronic disease or active infection were not included in the *Control group*.

Anthropometric measurements and assessment of nutritional status

Anthropometric measurements were obtained using standard techniques. For the assessment of growth, gender- and age-specific standard deviation score (SDS) of height were calculated according to the references for Turkish children [22]. Body mass index (BMI) was calculated using a patient's weight and height (kg/m^2). To estimate nutritional status, gender- and height-age-specific percentiles of BMI were used based on the national reference data [22]. Nutritional status was classified as underweight (< 5th percentile), normal-weight (5th to < 85th percentile), overweight (85th to < 95th percentile), and obese (≥ 95th percentile). Additionally, gender- and height-age-specific SDS of BMI was calculated for use in the definition of PEW.

Assessment of body composition

Body composition including fat mass and lean mass (kg) was estimated by multiple frequency bioimpedance analysis (BIA). Measurements were performed by the same physician using a portable body bioimpedance spectroscopy device (the Body Composition Monitor (BCM), Fresenius Medical Care, Germany). Fat mass index (FMI) and lean mass index (LMI) were calculated as the quotient of fat mass/height^2 and lean mass/height^2 (kg/m^2), respectively. Reduced muscle mass was defined as an LMI < 10th percentile of the *Control group* (LMI < 11.2 kg/m^2 for females and < 11.4 kg/m^2 for males).

All anthropometric measurements and body composition analyses were carried out at the same study visit and obtained in the morning after fasting at least 4 h, and after drainage of peritoneal fluid in PD patients, or 30 min after the mid-week session in HD patients.

Definition of PEW

The definition for PEW was modified from recently described criteria for clinical diagnosis of muscle-wasting syndromes in pediatric CKD [21]. According to the criteria, PEW is defined as having reduced body mass (BMI SDS < − 1.64 for gender- and height-age) plus at least two of the four following criteria: (1) poor growth (height SDS < − 1.88 for gender and age), (2) reduced muscle mass (LMI < 10th percentile), (3) plasma albumin concentration < 3.8 g/dL, and (4) decreased appetite or decreased dietary protein intake. Patients were questioned regarding their appetite using a simplified appetite questionnaire (SNAQ) [23]. A SNAQ score ≤ 14 was defined as poor appetite. For the assessment of dietary protein intake, protein catabolic rate (nPCR) was calculated by urea kinetic modeling in the HD patients [24] and obtained from peritoneal equilibrium test within the last 6 months in the PD patients. Poor protein intake was described as nPCR < 0.8 g/kg per day.

Laboratory assessments

Blood samples were drawn in the morning after fasting at least 4 h, just before a mid-week session in HD patients and after the first drainage of peritoneal fluid in PD patients. Blood samples of 3–5 mL in total were collected in EDTA containing tubes. The plasma was separated by centrifugation at 3000 rpm for 10 min and then immediately placed in a freezer and stored at − 80 °C for the measurement of leptin and total ghrelin. Plasma leptin (ng/mL) and total ghrelin (ng/mL) concentrations were measured by enzyme-linked immunosorbent assay (ELISA) using kits purchased from Assaypro (MO, USA) according to the manufacturer's instructions.

Plasma CRP levels were measured by high-sensitivity assays. Routine biochemical analyses were performed for the measurement of plasma urea, creatinine, and albumin. The updated Schwartz formula [25] was used for estimating glomerular filtration rate (eGFR). The homeostasis model assessment of insulin resistance (HOMA-IR) was calculated to estimate insulin resistance using fasting levels of plasma glucose (mg/dL) and insulin (mIU/L).

Statistical analysis

Statistical analysis was performed with the SPSS Statistics 20 version package program (SPSS, IBM Corporation, Chicago). Continuous data were expressed as median (25, 75%). Plasma leptin levels were logarithmically transformed to correct their skewed distributions before statistical analysis. The Kruskal-Wallis test was used for comparing continuous variables among more than two independent groups. Mann-Whitney *U* tests were used for the comparison of continuous variables between two independent groups. Categorical variables are presented as frequency (percentages), and the chi-squared test was used to compare two categorical variables. The Spearman's correlation coefficient was used to discover linear relationships for leptin and total ghrelin; the variables with *P* value of ≤ 0.1 were tested in a multivariate linear regression analysis. The level of statistical significance was defined as a two-tailed *P* value of < 0.05.

Results

A total of 94 patients were included in the study. The underlying primary renal diseases were congenital anomalies of the kidney and urinary tract ($n = 43$), glomerular disorders ($n = 15$), cystinosis ($n = 9$), neurogenic bladder ($n = 8$), hyperoxaluria ($n = 4$), non-neurogenic bladder ($n = 3$), tubulointerstitial disease ($n = 3$), autosomal recessive polycystic kidney disease ($n = 2$), hemolytic uremic syndrome ($n = 2$), and unknown origin ($n = 5$). None of the patients were on a specific diet program. All KTx recipients were receiving triple immunosuppressive therapy with prednisolone. None of the patients in the CKD or Dialysis groups were receiving steroid therapy. Only two KTx recipients were treated with growth hormone.

Demographic, clinical, and laboratory data of the study groups are summarized in Table 1. The Dialysis group had the lowest median LMI, FMI, and plasma albumin levels, as well as the highest median CRP levels among the study groups.

Leptin

Plasma leptin concentrations did not differ among the study groups ($P = 0.25$). The median levels of plasma leptin were 6.3 (2.8, 9.2), 4.5 (3.1, 16.5), 3.2 (1.7, 11.2), and 3.5 (2.5, 7.4) ng/mL in the Control, CKD, Dialysis, and KTx groups, respectively. Logarithmically transformed plasma leptin (log-leptin) levels are shown in Fig. 1a. There was no significant difference in log-leptin levels between females and males in any of the study groups. Besides plasma leptin levels, leptin to FMI ratio was also analyzed to correct the leptin levels for body fat mass. This ratio was significantly higher in the CKD group than in the Control group (Fig. 1b), but did not differ in Dialysis or KTx groups from the Control group. In the CKD group, leptin to FMI ratio was significantly higher in girls than in boys [2.8 (2.0, 6.1) vs 0.95 (0.6, 1.1) ng/mL/kg/m^2, $P = 0.009$).

Due to different clinical and nutritional characteristics of the patient groups, the univariate analyses of plasma leptin levels were performed separately and the results are shown in Table 2. Log-leptin levels were significantly and positively correlated with BMI-SDS, FMI, HOMA-IR, and CRP levels, and negatively correlated with eGFR in the CKD group, but none of these covariates remained as an independent predictor for leptin in the multivariate analysis. After adjustment for FMI, higher plasma leptin levels were independently associated with lower eGFR ($\beta = -0.422$, $P = 0.003$, CI 95% −0.055 to −0.012) and higher BMI-SDS ($\beta = 0.370$, $P = 0.008$, CI 95% 0.165 to 1.009). In the Dialysis group, leptin level was independently and positively associated with FMI ($\beta = 0.666$, $P < 0.001$, CI 95% 0.072 to 0.155) and BMI-SDS ($\beta = 0.256$, $P = 0.019$, CI 95% 0.021 to 0.219) (Fig. 2). In the KTx group, leptin level was independently and positively associated only with FMI ($\beta = 0.714$, $P = 0.001$, 95% CI 0.031 to 0.102). After adjustment for FMI, it was independently associated with BMI-SDS ($\beta = 0.394$, $P < 0.001$, CI 95% 0.304 to 0.983).

Ghrelin

The median total ghrelin level was significantly higher in the Dialysis group [1.33 (0.47, 2.59) ng/mL], compared to the Control group [0.39 (0.25, 0.75) ng/mL], CKD group [0.45

Table 1 Demographic, clinical, and laboratory data of the study groups

Variables	Control ($n = 18$)	CKD ($n = 20$)	Dialysis ($n = 39$)	KTx ($n = 35$)	P
Age, years	13.1 (11.5, 14.2)	14.6 (10.2, 16.9)	13.1 (10.1, 15.5)	15.0 (13.4, 18.3)	0.034[a]
Gender (female), n (%)	9 (50)	8 (40)	19 (49)	11 (31)	0.42
Height SDS	0.09 (− 0.40, 0.45)	− 1.04 (− 1.60, − 0.02)	− 2.67 (− 3.51, − 1.63)	− 2.61 (− 3.57, − 1.26)	< 0.001[b]
BMI SDS for height-age	0.35 (− 0.37, 1.10)	0.10 (− 1.16, 1.14)	− 0.13 (− 1.29, 0.89)	0.60 (− 0.06, 1.11)	0.16
FMI, kg/m^2	6.8 (4.9, 10.1)	4.6 (2.8, 7.7)	4.3 (3.3, 7.4)	7.2 (4.6, 10.2)	0.012[c]
LMI, kg/m^2	13.1 (12.3, 14.2)	12.6 (11.5, 14.5)	11.4 (10.3, 13.5)	11.8 (10.4, 13.0)	0.011[d]
Albumin, g/dL	4.9 (4.7, 5.0)	4.6 (4.2, 4.7)	4.0 (3.7, 4.3)	4.8 (4.6, 5.0)	< 0.001[e]
CRP, mg/dL	0.03 (0.03, 0.04)	0.09 (0.04, 0.24)	0.35 (0.31, 1.07)	0.08 (0.04, 0.20)	< 0.001[f]

Data presented as median (25, 75%) Kruskal Wallis test used for the comparison of the four groups (Mann-Whitney U test used for the comparison of the two groups; only significant differences are stated). None of the variables differed between PD and HD patients, except CRP levels. HD patients had significantly higher CRP levels than PD patients ($P = 0.042$)

CKD chronic kidney disease stages II–IV, *KTx* kidney transplant, *SDS* standard deviation score, *BMI* body mass index, *FMI* fat mass index, *LMI* lean mass index, *CRP* C-reactive protein

[a] Control vs KTx, $P = 0.009$; dialysis vs KTx, $P = 0.012$

[b] Control vs CKD, $P = 0.004$; control vs dialysis and control vs KTx, $P < 0.001$

[c] Control vs CKD, $P = 0.033$; control vs dialysis, $P = 0.035$; CKD vs KTx, $P = 0.020$; dialysis vs KTx, $P = 0.010$

[d] Control vs dialysis, $P = 0.005$; control vs KTx, $P = 0.007$; CKD vs dialysis, $P = 0.051$

[e] Control vs CKD, $P = 0.001$; control vs dialysis, CKD vs dialysis, and dialysis vs KTx, $P < 0.001$ (for each)

[f] Control vs dialysis, CKD vs dialysis, and dialysis vs KTx, $P < 0.001$ (for each)

(0.23, 0.63) ng/mL], and KTx group [0.28 (0.16, 0.66) ng/mL] ($P < 0.001$ for each group) (Fig. 3). There was no significant difference in total ghrelin levels between PD and HD patients [1.54 (0.50, 3.33) vs 1.00 (0.41, 1.62) ng/mL, $P = 0.08$].

Univariate correlation analyses showed that there was no relationship between total ghrelin levels and any of the clinical or laboratory variables in the Control, CKD, or KTx groups. In the Dialysis group, total ghrelin levels were negatively correlated with age ($r = -0.339$, $P = 0.035$), age at initiation of dialysis ($r = -0.384$, $P = 0.016$), BMI-SDS ($r = -0.395$, $P = 0.013$), FMI ($r = -0.460$, $P = 0.003$), and plasma albumin level ($r = -0.348$, $P = 0.030$). However, none of these variables remained an independent predictor of total ghrelin in the multivariate analysis. After adjustment for FMI, a high total ghrelin level was independently associated only with lower BMI-SDS in the Dialysis group ($\beta = -0.499$, $P = 0.001$, CI 95% − 0.269 to − 0.075). Additionally, an inverse relationship was found between leptin and ghrelin levels in this group ($r = -0.411$, $P = 0.009$).

Nutritional status

According to the classification of patients based on nutritional status, 12 out of 94 patients were classified as underweight; 8 of whom (20.5%) were in the Dialysis group, 3 (15%) in the CKD group, and 1 (3%) in the KTx group. A

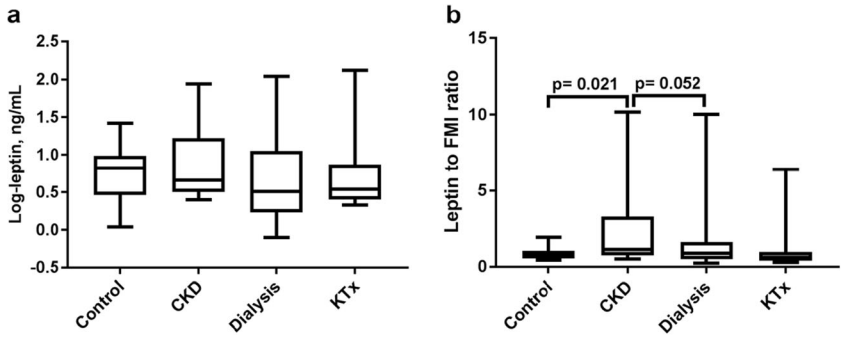

Fig. 1 Logarithmically transformed serum leptin (log-leptin) and the ratio of leptin to fat mass index (FMI) (ng/mL/kg/m^2) among the study groups (compared with Kruskal-Wallis test). Only significant differences between the two groups are shown on the figures (Mann-Whitney U test). **a** Log-leptin levels ($P = 0.25$). **b** Leptin to FMI ratio ($P = 0.003$)

Table 2 Univariate analysis of *plasma leptin* levels in the study groups

	Leptin (ng/dL)[a]							
	Control		CKD		Dialysis		KTx	
	r	*p*	*r*	*p*	*r*	*p*	*r*	*p*
Age, years	− 0.207	0.41	0.358	0.13	0.091	0.58	0.580	< 0.001
Dialysis vintage, months					− 0.366	0.022	− 0.212	0.24
BMI, kg/m^2	0.744	< 0.001	0.668	0.002	0.693	< 0.001	0.603	< 0.001
BMI-SDS for height-age	0.649	0.004	0.751	< 0.001	0.783	< 0.001	0.329	0.054
FMI, kg/m^2	0.858	< 0.001	0.798	< 0.001	0.769	< 0.001	0.683	< 0.001
LMI, kg/m^2	− 0.022	0.93	0.279	0.25	− 0.123	0.46	− 0.231	0.18
Albumin, g/dL	0.038	0.88	− 0.318	0.19	0.456	0.004	0.009	0.96
HOMA-IR			0.596	0.019	0.572	< 0.001	0.459	0.007
CRP, mg/dL	− 0.582	0.011	0.513	0.035	0.085	0.61	0.203	0.24
eGFR, mL/min/1.73 m^2	0.370	0.13	− 0.489	0.033			− 0.242	0.16

Spearman's correlation test

CKD chronic kidney disease stages II–IV, *KTx* kidney transplant, *BMI* body mass index, *SDS* standard deviation score, *FMI* fat mass index, *LMI* lean mass index, *HOMA-IR* homeostasis model assessment of insulin resistance, *CRP* C-reactive protein, *eGFR* estimated glomerular filtration rate

[a] Logarithmically transformed plasma leptin levels used for statistical analyses

total of 16 patients were overweight (4 in CKD, 6 in Dialysis, and 6 in KTx group), and 8 were obese (1 in CKD, 2 in Dialysis, and 5 in KTx group). The remaining 58 patients had normal weight.

The median plasma leptin concentrations were 1.45 (1.19, 2.39), 2.50 (2.02, 3.23), 3.26 (2.54, 10.1), and 34.1 (11.3, 88.9) ng/mL in underweight, normal-weight, overweight, and obese patients, respectively. There was a significant difference in median log-leptin concentrations between the groups regarding nutritional status ($P < 0.001$), with the lowest levels in underweight patients and the highest levels in obese patients (Fig. 4a). Leptin to FMI ratio was significantly higher in overweight and obese patients compared to the normal-weight patients ($P = 0.048$ and $P = 0.027$, respectively); however, any difference in this ratio was not observed between underweight and normal weight patients (Fig. 4b). Plasma total ghrelin concentrations did not differ among the groups based on nutritional status (Fig. 4c).

PEW

A total of seven dialysis patients (18%) (five HD and two PD patients) and one CKD patient (5%), but none of the KTx recipients, met the criteria of PEW. Only dialysis patients were evaluated with respect to PEW. Dialysis patients with PEW had significantly lower median BMI-SDS for height-age [− 2.18 (− 2.38, − 1.72) vs 0.34 (− 0.75, 1.18), $P < 0.001$] as well as lower LMI [10.6 (9.46, 10.8) vs 11.6 (10.6, 13.7) kg/m^2, $P = 0.014$] and lower FMI [2.90 (2.00, 3.60) vs 5.18 (3.65, 9.02) kg/m^2, $P = 0.003$] (Fig. 5a, b). Patients with PEW also had lower log-leptin levels compared to their counterparts [0.23 (0.14, 0.49) vs 0.56 (0.35, 1.29) ng/mL, $P = 0.018$] (Fig. 5c); however, there was no significant difference in the ratio of leptin to FMI between the two groups ($P = 0.19$). Total ghrelin levels did not differ between the patients with and without PEW ($P = 0.10$) (Fig. 5d). No differences were found between the two groups

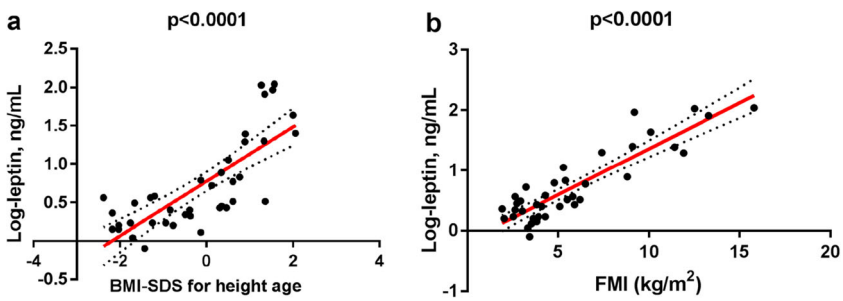

Fig. 2 The relationships of logarithmically transformed leptin (log-leptin) in dialysis patients. **a** Body mass index (BMI)-SDS for height age. **b** Fat mass index (FMI)

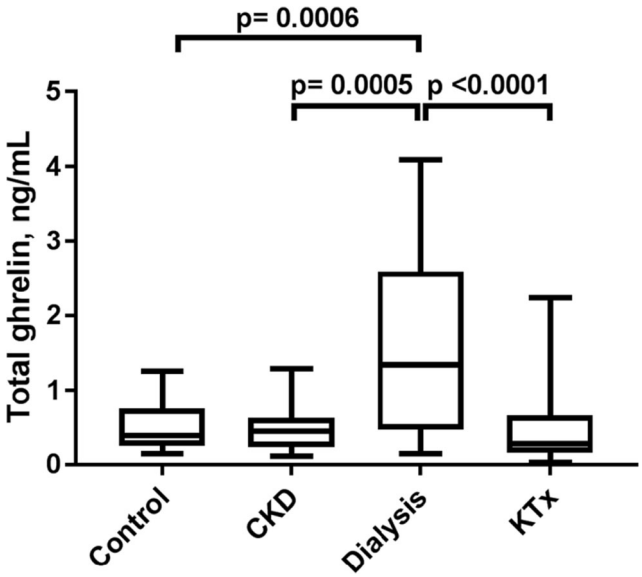

Fig. 3 Total ghrelin levels in the study groups ($P < 0.001$) (Kruskal-Wallis test). Only significant differences between the two groups are shown on the figures (Mann-Whitney *U* test). *CKD* chronic kidney disease, *KTx* kidney transplant

considering age, gender, dialysis vintage, type of dialysis, kt/V, nPCR, plasma albumin, or CRP levels.

Discussion

This study presents plasma concentrations of leptin and total ghrelin in relation to BIA-based body fat mass in three different pediatric CKD groups (CKD stages II–IV, Dialysis, and KTx), especially focusing on PEW according to the recently described criteria in children with CKD. The present study demonstrates that patients with CKD stages II–IV have disproportionally high levels of plasma leptin after correction for body fat mass, whereas dialysis patients have no alterations in plasma leptin levels, even after correction for fat mass. This study also demonstrates a significant elevation in plasma total ghrelin levels which is associated with lower BMI-SDS in dialysis patients. Lastly, the current study highlights that PEW is prevalent in dialysis with 18% of dialysis patients suffering from PEW. Dialysis patients with PEW have significantly decreased plasma leptin concentrations, and fat mass is the most important independent regulator of plasma leptin concentrations in this group.

Controversial data have been reported in the literature regarding leptin concentrations in CKD and ESKD patients on dialysis. Some studies, mostly conducted in the adult population, have revealed hyperleptinemia [6–8, 26–28], while several pediatric studies have demonstrated no alteration in plasma levels of this hormone in patients with CKD [12–14]. Our study shows no difference in plasma leptin levels among different CKD groups including CKD stages II–IV, Dialysis, or KTx compared to the healthy controls. Leptin is mainly secreted into the blood stream from the adipose tissue, and body fat mass is considered to be the main determinant of plasma leptin concentrations [29]. Hence, we analyzed not only plasma leptin levels but also the leptin corrected for BIA-based fat mass. Patients with CKD stages II–IV but not dialysis patients had disproportionally elevated plasma leptin concentrations in relation to body fat mass. This finding is in line with the studies demonstrating a significant elevation in plasma leptin levels corrected for fat mass both in children [30] and adults with CKD [31].

The exact cause of hyperleptinemia in CKD is not clear. The kidneys are responsible for the clearance of leptin by glomerular filtration followed by metabolic degradation in the renal tubules [32]. Elevated leptin levels in uremic patients are considered to be associated with reduced renal function which leads to the accumulation of this hormone [8, 10, 30]; however, the results of the CKiD cohort reported by Nehus et al. [33] have suggested that the renal clearance of leptin does not contribute to its elevated levels. Our results show that

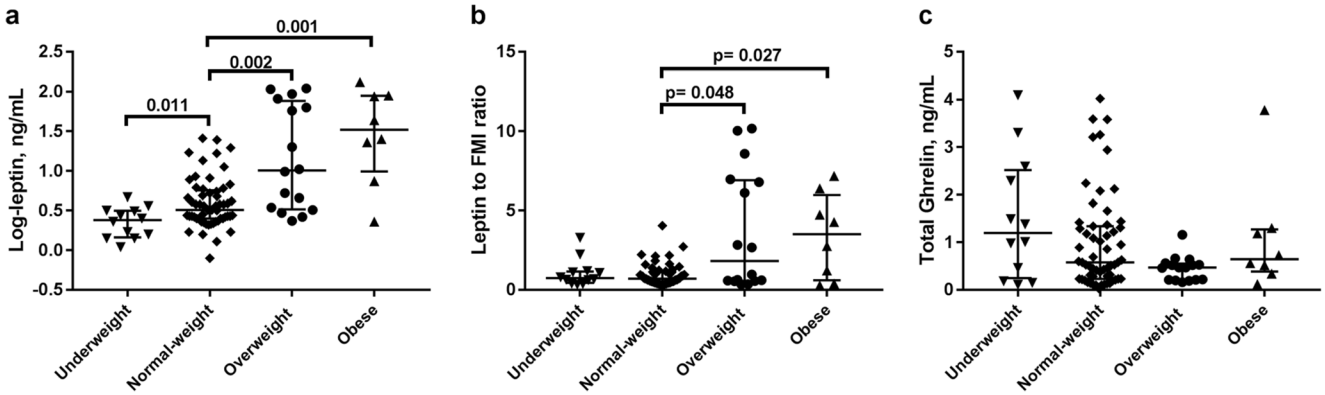

Fig. 4 Comparisons of logarithmically transformed serum leptin (log-leptin), leptin to fat mass index (FMI) ratio (ng/mL/kg/m^2), and serum total ghrelin levels among the groups classified regarding to nutritional status (Kruskal-Wallis test). Only significant differences between the two groups are shown on the figures (Mann-Whitney *U* test). **a** Log-leptin levels ($P < 0.0001$). **b** Leptin to FMI ratio ($P = 0.06$). **c** Total ghrelin levels ($P = 0.20$)

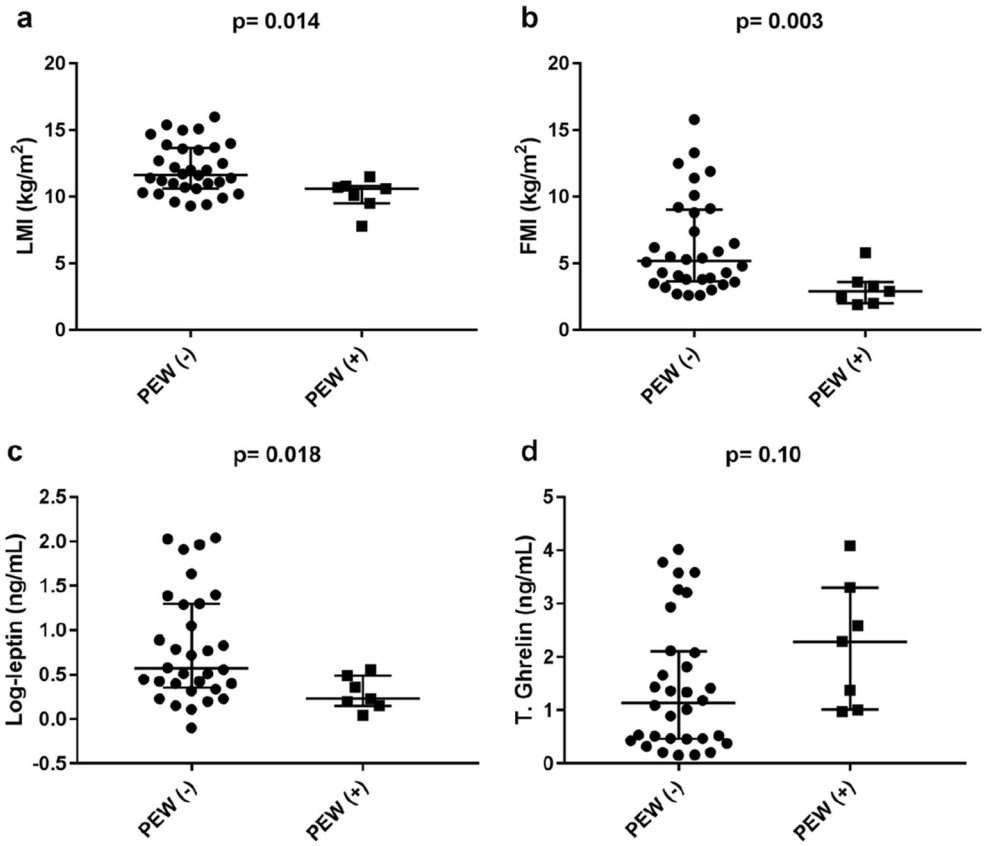

Fig. 5 Comparisons of the dialysis patients with and without protein-energy wasting (PEW) (Mann-Whitney *U* test). **a** Lean mass index (LMI). **b** Fat mass index (FMI). **c** Logarithmically transformed plasma leptin (log-leptin) levels [the median plasma leptin concentrations (original levels not logarithmically) were 1.7 (1.4, 3.1) and 3.7 (2.3, 19.7) ng/mL in patients with and without PEW]. **d** Total ghrelin levels

eGFR is an independent risk factor for elevated leptin levels after correction for fat mass in patients with CKD stages II–IV. This finding supports the idea that decreased renal clearance contributes to elevated circulating levels of leptin in patients with CKD. It is also suggested that insufficient renal clearance of leptin by PD or low-flux HD results in elevation of plasma leptin levels in dialysis patients [8, 34]; however, high-flux HD and hemodiafiltration significantly reduce plasma levels of leptin [35, 36]. Increased production of leptin may contribute to the plasma concentrations of leptin in dialysis patients [6, 37]. In a pediatric study conducted by Büscher et al. [13], it was demonstrated that uremic patients had unaltered leptin levels which were most probably a result of reduced production due to their lower BMI-SDS. Consistent with this finding, our study showed unaltered plasma levels of leptin in dialysis patients who had the worst nutritional status, with the lowest lean and fat mass and lowest serum albumin levels compared to the other patient groups, as well as having the highest prevalence of PEW. Our study also demonstrated a strong positive association between plasma leptin levels and fat mass. Taken together, the unexpectedly unaltered leptin levels in dialysis patients can be explained by the reduced production of leptin from the reduced fat mass.

There is increasing evidence supporting a more complex role of ghrelin in the regulation of body weight much more than a hunger hormone [15]. Circulating ghrelin exists in two major forms that have opposite effects on food intake. While the acylated form of ghrelin has potent orexigenic effect and stimulates food intake, the des-acyl form of ghrelin shows anorexigenic activity [38]. Ghrelin is primarily degraded by the kidney, and its concentration is elevated by declining renal function [39]. Plasma levels of total and des-acyl ghrelin, but not acyl ghrelin, increase in patients with CKD, particularly in patients with ESKD on maintenance dialysis [12–14, 16, 18, 40]. Total ghrelin predominantly represents the des-acyl form of ghrelin which has an anorexigenic effect and suppresses food intake. The acylation is essential for its orexigenic and adipogenic effects [38, 41], and ghrelin activation is suggested to be impaired in patients with CKD [14]. Low plasma levels of acyl ghrelin have been linked to malnutrition and poor appetite in adult HD patients [42]. On the other hand, increase in the anorexigenic form des-acyl ghrelin is considered to be associated with poor appetite, low energy intake, and PEW in both adult and pediatric dialysis patients [13, 18, 43]. Our study reveals a significant elevation in plasma total ghrelin levels in children on chronic dialysis predicted by a low BMI-SDS for height-age. This finding is consistent with the previous pediatric studies [13, 18] that may indicate a relationship between elevated plasma levels of total ghrelin and poor nutritional status. However, we were not able to show any difference in total ghrelin levels between the patients with and without PEW. It is important to point out that we

unfortunately used the assay for total ghrelin that is the sum of the des-acyl and acyl forms of ghrelin, but also detects inactive ghrelin fragments. Due to their opposite effects on energy metabolism, assessment of these two forms separately using specific assays would have been very helpful to identify the causal effect of ghrelin on the development of PEW in children undergoing dialysis in a large cohort.

PEW is prevalent, ranging from 18 to 75% in adult patients on maintenance dialysis [44]. Dysregulation in appetite-regulating hormones is one of the contributing factors for this complex and multifactorial condition [20]. Although the co-existence of elevated leptin levels with uremia is mostly interpreted as an evidence of the cause of CKD-related PEW, several studies have shown that plasma leptin levels positively correlate with BMI and fat mass [9, 33, 45]. Our study also shows a strong positive relationship between leptin levels and BMI-SDS, suggesting the association of leptin with a favorable nutritional status. Additionally, several aforementioned pediatric studies have not documented high leptin levels in dialysis patients. We found no evidence of elevated plasma leptin levels in dialysis patients; we even found low levels of leptin in dialysis patients with PEW. In our study, PEW was classified according to the criteria which include anthropometric measurements (height SDS and BMI SDS) as well as BIA-based lean mass, serum albumin levels, and protein intake (nPCR in dialysis patients). It is also important to note that we used height-adjusted SDS for BMI as recommended by the KDOQI guideline [46]. According to this classification, 18% of the dialysis patients in our cohort had PEW. Those patients with PEW not only had significantly lower BMI-SDS and lower lean mass but also significantly lower fat mass and lower leptin levels compared to the dialysis patients without PEW. Considering a strong relationship between leptin levels and fat mass in this cohort, decrease in fat mass may explain low levels of plasma leptin in patients with PEW. Our result therefore suggests that low levels of leptin may be a consequence rather than a cause of PEW.

Because leptin is known as an anorexigenic hormone, the question is why low leptin levels do not lead to an increase in appetite in our cohort. In fact, low leptin levels are expected to stimulate appetite via leptin signaling in the hypothalamus which is the main target of leptin. On the other hand, it is also thought that this mechanism is not preserved in most chronic diseases [47]. Indeed, it is stated that leptin levels are significantly decreased in other cachexia syndromes, such as cancer-cachexia and pulmonary cachexia, and this decrease in leptin is not associated with a compensatory increase in appetite [47]. A similar paradox exists in obesity. Although obese patients have high plasma leptin concentrations due to their excess fat mass, the appetite of obese patients is not suppressed. This condition has been interpreted as leptin resistance explaining with the impairment in leptin transportation and leptin signaling in the hypothalamus [48]. These results suggest that leptin has a complex network between central and peripheral signaling that can be altered in chronic conditions. Therefore, the role of leptin in the pathogenesis of PEW has been difficult to interpret in the CKD population, and further studies are needed to deepen our understanding.

The most important strength of the present study is the evaluation of the actions of leptin and ghrelin hormones according to the presence of PEW as defined using newly described criteria. The other strength of our study is, unlike other pediatric studies, it uses the height-adjusted SDS of BMI for the assessment of nutritional status. Additionally, this study evaluates the hormone levels in relation to fat and lean mass of the patients. One of the most important limitations of the present study is the relatively small cohort as compared to adult studies. The power of the statistical analysis could be increased by increasing sample size. Assessment of only total ghrelin instead of specific assays for different forms of ghrelin (acylated vs deacylated) is another limitation of the study. Our study was also limited by the cross-sectional design, which precludes definition of causal relationships between these two hormones and PEW. To clarify the result of low leptin levels in PEW, longitudinal studies with a larger sample size of pediatric dialysis patients are needed.

In conclusion, we have shown that PEW is common in dialysis patients. Our results suggest that higher plasma total ghrelin concentrations may be an indicator for poor nutritional status in dialysis patients. On the other hand, our study shows that both underweight patients in the whole cohort, as well as dialysis patients with PEW, have lower levels of leptin associated with decreased fat mass. The effect of leptin in the regulation of appetite and the development of PEW appears to be complex in uremia. Further studies are needed to discern the cause and effect relationships between leptin and PEW.

Acknowledgements This work was supported by Scientific Research Projects Coordination Unit of Istanbul University (project number 35291).

Compliance with ethical standards

The study protocol was approved by the Ethical Committee of Istanbul University, Cerrahpasa Faculty of Medicine.

Conflict of interest The authors declare that they have no conflicts of interest.

References

1. Mak RH, Ikizler AT, Kovesdy CP, Raj DS, Stenvinkel P, Kalantar-Zadeh K (2011) Wasting in chronic kidney disease. J Cachexia Sarcopenia Muscle 2:9–25
2. Mak RH, Cheung WW, Zhan JY, Shen Q, Foster BJ (2012) Cachexia and protein-energy wasting in children with chronic kidney disease. Pediatr Nephrol 27:173–181

3. Gunta SS, Mak RH (2013) Ghrelin and leptin pathophysiology in chronic kidney disease. Pediatr Nephrol 28:611–616
4. Klok MD, Jakobsdottir S, Drent ML (2007) The role of leptin and ghrelin in the regulation of food intake and body weight in humans: a review. Obes Rev 8:21–34
5. Young GA, Woodrow G, Kendall S, Oldroyd B, Turney JH, Brown-John AM, Smith MA (1997) Increased plasma leptin/fat ratio in patient with chronic renal failure: a cause of malnutrition? Nephrol Dial Transplant 12:2318–2323
6. Heimbürger O, Lönnqvist F, Danielsson A, Nordenström J, Stenvinkel P (1997) Serum immunoreactive leptin concentration and its relation to the body fat content in chronic renal failure. J Am Soc Nephrol 8:1423–1430
7. Merabet E, Dagogo-Jack S, Coyne DW, Klein S, Santiago JV, Hmiel SP, Landt M (1997) Increased plasma leptin concentration in end-stage renal disease. J Clin Endocrinol Metab 82:847–850
8. Sharma K, Considine RV, Michael B, Dunn SR, Weisberg LS, Kurnik BR, Kurnik PB, O'Connor J, Sinha M, Caro JF (1997) Plasma leptin is partly cleared by the kidney and is elevated in hemodialysis patients. Kidney Int 51:1980–1985
9. Stenvinkel P, Lindholm B, Lönnqvist F, Katzarski K, Heimbürger O (2000) Increases in serum leptin levels during peritoneal dialysis are associated with inflammation and a decrease in lean body mass. J Am Soc Nephrol 11:1303–1309
10. Shankar A, Syamala S, Xiao J, Muntner P (2012) Relationship between plasma leptin level and chronic kidney disease. Int J Nephrol 2012:269532
11. Nishikawa M, Takagi T, Yoshikawa N, Shouzu A, Murakami T, Kono M, Owae H, Tanaka K, Iwasaka T, Inada M (1999) Measurement of serum leptin in patients with chronic renal failure on hemodialysis. Clin Nephrol 51:296–303
12. Arbeiter AK, Büscher R, Petersenn S, Hauffa BP, Mann K, Hoyer PF (2009) 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
13. Büscher AK, Büscher R, Hauffa BP, Hoyer PF (2010) Alterations in appetite-regulating hormones influence protein-energy wasting in pediatric patients with chronic kidney disease. Pediatr Nephrol 25: 2295–2301
14. Naufel MF, Bordon M, de Aquino TM, Ribeiro EB, de Abreu Carvalhaes JT (2010) Plasma levels of acylated and total ghrelin in pediatric patients with chronic kidney disease. Pediatr Nephrol 25:2477–2482
15. Müller TD, Nogueiras R, Andermann ML, Andrews ZB, Anker SD, Argente J, Batterham RL, Benoit SC, Bowers CY, Broglio F, Casanueva FF, D'Alessio D, Depoortere I, Geliebter A, Ghigo E, Cole PA, Cowley M, Cummings DE, Dagher A, Diano S, Dickson SL, Diéguez C, Granata R, Grill HJ, Grove K, Habegger KM, Heppner K, Heiman ML, Holsen L, Holst B, Inui A, Jansson JO, Kirchner H, Korbonits M, Laferrère B, LeRoux CW, Lopez M, Morin S, Nakazato M, Nass R, Perez-Tilve D, Pfluger PT, Schwartz TW, Seeley RJ, Sleeman M, Sun Y, Sussel L, Tong J, Thorner MO, van der Lely AJ, van der Ploeg LH, Zigman JM, Kojima M, Kangawa K, Smith RG, Horvath T, Tschöp MH (2015) Ghrelin. Mol Metab 4:437–460
16. Gupta RK, Kuppusamy T, Patrie JT, Gaylinn B, Liu J, Thorner MO, Bolton WK (2013) Association of plasma des-acylghrel in levels with CKD. Clin J Am Soc Nephrol 8:1098–1105
17. Cheung WW, Mak RH (2010) Ghrelin in chronic kidney disease. Int J Pept. https://doi.org/10.1155/2010/567343
18. Monzani A, Perrone M, Prodam F, Moia S, Genoni G, Testa S, Paglialonga F, Rapa A, Bona G, Montini G, Edefonti A (2018) Unacylated ghrelin and obestatin: promising biomarkers of protein energy wasting in children with chronic kidney disease. Pediatr Nephrol 33:661–672
19. Fouque D, Kalantar-Zadeh K, Kopple J, Cano N, Chauveau P, Cuppari L, Franch H, Guarnieri G, Ikizler TA, Kaysen G, Lindholm B, Massy Z, Mitch W, Pineda E, Stenvinkel P, Trevino-Becerra A, Wanner C (2008) A proposed nomenclature and diagnostic criteria for protein-energy wasting in acute and chronic kidney disease. Kidney Int 73:391–398
20. Carrero JJ, Stenvinkel P, Cuppari L, Ikizler TA, Kalantar-Zadeh K, Kaysen G, Mitch WE, Price SR, Wanner C, Wang AY, ter Wee P, Franch HA (2013) Etiology of the protein-energy wasting syndrome in chronic kidney disease: a consensus statement from the International Society of Renal Nutrition and Metabolism (ISRNM). J Ren Nutr 23:77–90
21. Oliveira EA, Cheung WW, Toma KG, Mak RH (2018) Muscle wasting in chronic kidney disease. Pediatr Nephrol 33:789–798
22. Neyzi O, Bundak R, Gökçay G, Günöz H, Furman A, Darendeliler F, Baş F (2015) Reference values for weight, height, head circumference, and body mass index in Turkish children. J Clin Res Pediatr Endocrinol 7:280–293
23. Wilson MM, Thomas DR, Rubenstein LZ, Chibnall JT, Anderson S, Baxi A, Diebold MR, Morley JE (2005) Appetite assessment: simple appetite questionnaire predicts weight loss in community-dwelling adults and nursing home residents. Am J Clin Nutr 82: 1074–1081
24. Jindall KK, Goldstein MB (1988) Urea kinetic modeling in chronic hemodialysis: benefits, problems, and practical solutions. Semin Dial 1:82
25. Schwartz GJ, Muñoz A, Schneider MF, Mak RH, Kaskel F, Warady BA, Furth SL (2009) New equations to estimate GFR in children with CKD. J Am Soc Nephrol 20:629–637
26. Wolf G, Chen S, Han DC, Ziyadeh FN (2002) Leptin and renal disease. Am J Kidney Dis 39:1–11
27. Besbas N, Ozaltin F, Coşkun T, Ozalp S, Saatçi U, Bakkaloğlu A, El Nahas AM (2003) Relationship of leptin and insulin-like growth factor I to nutritional status in hemodialyzed children. Pediatr Nephrol 18:1255–1259
28. Buyan N, Bideci A, Ozkaya O, Ortac E, Bakkaloglu S, Gonen S, Peru H, Soylemezoglu O, Cinaz P (2006) Leptin and resistin levels and their relationships with glucose metabolism in children with chronic renal insufficiency and undergoing dialysis. Nephrology 11:192–196
29. Nagy TR, Gower BA, Trowbridge CA, Dezenberg C, Shewchuk RM, Goran MI (1997) Effects of gender, ethnicity, body composition, and fat distribution on serum leptin concentrations in children. J Clin Endocrinol Metab 82:2148–2152
30. Daschner M, Töshöff B, Blum WF, Englaro P, Wingen AM, Schaefer F, Wühl E, Rascher W, Mehls O (1998) Inappropriate elevation of serum leptin levels in children with chronic kidney disease. J Am Soc Nephrol 9:1074–1079
31. Stenvinkel P, Heimbürger O, Lönnqvist F (1997) Serum leptin concentrations correlate to plasma insulin concentrations independent of body fat content in chronic renal failure. Nephrol Dial Transplant 12:1321–1325
32. Cumin F, Baum HP, de Gasparo M, Levens N (1997) Removal of endogenous leptin from the circulation by the kidney. Int J Obes Relat Metab Disord 21:495–504
33. Nehus E, Furth S, Warady B, Mitsnefes M (2014) Correlates of leptin in children with chronic kidney disease. J Pediatr 165:825–829
34. Fontán MP, Rodríguez-Carmona A, Cordido F, García-Buela J (1999) Hyperleptinemia in uremic patients undergoing conservative management, peritoneal dialysis, and hemodialysis: a comparative analysis. Am J Kidney Dis 34:824–831
35. Wiesholzer M, Harm F, Hauser AC, Pribasnig A, Balcke P (1998) Inappropriately high plasma leptin levels in obese haemodialysis patients can be reduced by high flux haemodialysis and haemodiafiltration. Clin Sci (Lond) 94:431–435

36. Coyne DW, Dagogo-Jack S, Merabet E, Audrian J, Landt M (1998) High-flux dialysis lowers plasma leptin concentration in chronic dialysis patients. Am J Kidney Dis 32:1031–1035
37. Landt M, Parvin CA, Dagogo-Jack S, Bryant B, Coyne D (1999) Leptin elimination in hyperleptinaemic peritoneal dialysis patients. Nephrol Dial Transplant 14:732–737
38. Delporte C (2013) Structure and physiological actions of ghrelin. Scientifica (Cairo) 2013:518909
39. Yoshimoto A, Mori K, Sugawara A, Mukoyama M, Yahata K, Suganami T, Takaya K, Hosoda H, Kojima M, Kangawa K, Nakao K (2002) Plasma ghrelin and des-acylghrelin concentrations in renal failure. J Am Soc Nephrol 13:2748–2752
40. Rodriguez Ayala E, Pecoits-Filho R, Heimbürger O, Lindholm B, Nordfors L, Stenvinkel P (2004) Associations between plasma ghrelin levels and body composition in end-stage renal disease: a longitudinal study. Nephrol Dial Transplant 19:421–426
41. Delhanty PJ, Neggers SJ, van der Lely AJ (2012) Mechanisms in endocrinology: ghrelin: the differences between acyl- and des-acylghrelin. Eur J Endocrinol 167:601–608
42. Sharma R, Agrawal S, Saxena A, Pandey M, Sharma RK (2013) Association of genetic variants of ghrelin, leptin and UCP2 with malnutrition inflammation syndrome and survival in end-stage renal disease patients. Genes Nutr 8:611–621
43. Caliskan Y, Yelken B, Gorgulu N, Ozkok A, Yazici H, Telci A, Turkmen A, Yildiz A, Sever MS (2012) Comparison of markers of appetite and inflammation between hemodialysis patients with and without failed renal transplants. J Ren Nutr 22:258–267
44. Kalantar-Zadeh K, Kopple JD (2001) Relative contributions of nutrition and inflammation to clinical outcome in dialysis patients. Am J Kidney Dis 38:1343–1350
45. Chudek J, Adamczak M, Kania M, Hołowiecka A, Rozmus W, Kokot F, Wiecek A (2003) Does plasma leptin concentration predict the nutritional status of hemodialyzed patients with chronic renal failure? Med Sci Monit 9:CR377–CR382
46. KDOQI Work Group (2009) KDOQI clinical practice guideline for nutrition in children with CKD: 2008 update. Am J Kidney Dis 53(Suppl 2):S1–S124
47. Engineer DR, Garcia JM (2012) Leptin in anorexia and cachexia syndrome. Int J Pept. https://doi.org/10.1155/2012/287457
48. Zhou Y, Rui L (2013) Leptin signaling and leptin resistance. Front Med 7:207–222