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Protein-energy wasting modifies the association of ghrelin with inflammation, leptin, and mortality in hemodialysis patients

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Ghrelin abnormalities contribute to anorexia, inflammation, and cardiovascular risk in hemodialysis patients, leading to worse outcome. However, ghrelin levels are influenced by the nutritional status of the individual. We hypothesized that the consequences of ghrelin alterations in hemodialysis patients are context sensitive and dependent on the presence of protein-energy wasting (PEW). In this cross-sectional study of 217 prevalent hemodialysis patients followed for 31 months, we measured ghrelin, leptin, PEW (subjective global assessment), and C-reactive protein (an index of inflammation). Compared to patients in the middle and upper tertile of ghrelin levels, those in the lowest tertile were older, had higher leptin levels and body mass index, and presented an increased mortality risk that persisted after adjustment for age, gender, and dialysis vintage. This risk was lost after correction for comorbidities. Patients with PEW and low ghrelin values had abnormally high C-reactive protein and leptin by multivariate analysis of variance, and the highest mortality risk compared to non-PEW with high ghrelin from all-cause and cardiovascular-related mortality (adjusted hazard ratios of 3.34 and 3.54, respectively). Low ghrelin values in protein-energy wasted hemodialysis patients were linked to a markedly increased cardiovascular mortality risk. Thus, since these patients were more anorectic, our results provide a clinical scenario where ghrelin therapies may be particularly useful.

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The prevalence of protein-energy wasting (PEW), manifested as a loss of muscle mass and a mismatch between energy expenditure and intake, is high in advanced chronic kidney disease (CKD).[1–3] Like in other wasted patient groups, anorexia is common and often linked to persistent systemic inflammation, reduced quality of life, and increased mortality.[4,5] The regulation of anorexia includes a complex hypothalamic process in which different appetite-regulating centers are affected not only by neuropeptides, but also by peripheral signals from fat tissue and the gut.[6–8]

Ghrelin is an orexigenic peptide released primarily from endocrine cells in the stomach, which increases appetite and adjusts both short-term and long-term energy balance.[9] The orexigenic effects of ghrelin are mediated through the type 1a growth hormone secretagogue receptor, leading to increased gene expression of orexigenic neuropeptides and increased growth hormone (GH) release.[10] In advanced CKD, total ghrelin levels are high[11,12]—a finding that seems counter-intuitive given its orexigenic action and that has been interpreted as a defense mechanism against starvation. Yet, and despite this elevation/resistance, subcutaneous ghrelin administration resulted in several-fold increases in plasma ghrelin concentration followed by improvements in short-term energy intake and energy balance in mildly to moderately malnourished dialysis patients.[13,14] Similarly, a superagonist of GH-releasing hormone caused rapid improvement of nutritional status in CKD stage 4 and 5 patients without apparent GH deficiency.[15] Furthermore, ghrelin appears to be involved in other pathophysiological pathways such as improvement of cardiac function,[16,17] suppression of sympathetic activity,[18] inhibition of the inflammatory response,[19,20] anabolic effects on lean mass,[21,22] metabolic syndrome,[23] and mediation in insulin sensitivity signaling[24] or atherosclerosis.[25] In CKD, all these pathways have also been linked to PEW.[1]

Several studies, both in animals and humans, have suggested that not only is ghrelin dependent on body fat mass,[26] but is also influenced by the individual's nutritional status; although the orexigenic effect of peripheral ghrelin

administration differed between rats with different baseline food intake,[27] ghrelin values were markedly different among women with anorexia nervosa and constitutionally thin women, who display a similar low body mass index (BMI) but no nutritional disorder.[28,29] In advanced CKD, PEW is a common problem, representing severe and complex processes of muscle loss, poor food intake, inflammation and cardiovascular disease (CVD)[1] pathways, all of which share intriguing links with the purported ghrelin actions discussed above. Interestingly, the combined effect of ghrelin and higher food intake, but not ghrelin alone, was able to enhance skeletal muscle mitochondrial oxidative capacity and AKT phosphorylation in rats with CKD.[30] Given the interrelations of PEW with ghrelin, we hypothesized that the implications of low ghrelin in CKD patients are context sensitive and dependent on the presence of PEW. With this purpose, we assessed total ghrelin in a well-characterized cohort of 217 prevalent patients undergoing hemodialysis.

RESULTS

The study population consisted of 217 patients undergoing hemodialysis (125 men; 57%) with a median age of 66 (25th–75th percentile 51–74) years. The patients had an average BMI of $24.5 \pm 5.2\,kg/m^2$. Of these patients, 55 (25%) had diabetes, 139 (36%) had clinical signs or history of CVD, and 102 (47%) were wasted (subjective global assessment (SGA) >1). Patients underwent hemodialysis three times weekly (4 to 5 h per session) using bicarbonate dialysate. They had undergone hemodialysis for a median period of 29 months (15–58) months and the majority was anuric. Most patients used polyamide membranes (59%), followed by polysulfone (35%). Regarding vascular access, 58% had an arteriovenous fistula, whereas 22 and 20% had grafts and central dialysis catheters, respectively.

The general characteristics of the patients according to ghrelin thirds (low third vs the other two-thirds combined) are summarized in Table 1. We should remind the reader that, for a correct interpretation of our results, our definitions of low and high ghrelin correspond to the patients' range. Patients with low ghrelin levels were older, had higher BMI, higher plasma levels of leptin, lower plasma levels of adiponectin, and tended to be more often males. Table 1 also shows the univariate associations between ghrelin levels and selected variables as assessed by Spearman's rank test. Ghrelin concentration positively associated with adiponectin, whereas negatively associated with age, male sex, BMI, and leptin (as well as the leptin/BMI ratio).

Survival analysis was determined after a median follow-up period of 31 (20–38) months. During this period, 83 (38%) deaths occurred, of which 36 (44% of all deaths) were because of purportedly CVD-related causes. The impact of ghrelin levels on outcome was studied by the Kaplan–Meier method using the high ghrelin group (middle and high thirds combined) as the reference. Patients with low ghrelin levels had a worse all-cause mortality (log-rank (χ^2) 5.50; $P = 0.01$). Crude and adjusted Cox proportional hazard ratios (HRs) for mortality showed that patients with low ghrelin values had a significant crude HR (compared with patients with high ghrelin) of 1.68 (95% confidence interval (CI) 1.08–2.60) that persisted after adjustment of age, sex, and dialysis vintage (HR 1.55, 95% CI 0.99–2.40), but disappeared after further adjustment for comorbidities.

We then studied the implications of low ghrelin levels in the context of PEW. The clinical and biochemical

Table 1 | General characteristics according to ghrelin thirds and univariate associations with serum ghrelin concentration in 217 hemodialysis patients[a]

	Low ghrelin (n=72)	High ghrelin (n=145)	P-value[b]	ρ[c]
Ghrelin, pg/ml	231 (173–261)	423 (367–561)	—	—
Age, years	69 (55–80)[d]	63 (50–72)	0.006	−0.17**
Men, %	67[e]	53	0.05	—
Dialysis vintage, months	30 (15–55)	28 (14–58)	0.9	−0.02
Diabetes, %	29.2	23.4	0.3	—
CVD, %	66.7	62.8	0.6	—
PEW[f], %	44.4	48.3	0.6	—
BMI, kg/m^2	25.6 ± 4.8[g]	24.0 ± 5.3	0.01	−0.26***
Total cholesterol, mmol/l	4.3 ± 1.1	4.4 ± 1.0	0.7	0.09
Serum albumin, g/l	34.2 ± 4.6	34.9 ± 4.4	0.4	0.02
CRP, mg/l	6.5 (2.9–17.0)	7.0 (2.5–22.5)	0.9	0.07
Nt-Pro-BNP, pg/l	8.4 (3.3–21.7)	7.3 (2.8–33.9)	0.8	0.02
Adiponectin, μg/ml	19.2 (11.9–26.2)	23.9 (15.4–32.7)	0.001	0.34***
Leptin, ng/ml	19.9 (8.4–64.4)	13.4 (5.1–44.2)	0.01	−0.23***
Leptin/BMI	0.81 (0.40–2.22)	0.58 (0.24–1.90)	0.02	−0.20**

Abbreviations: BMI, body mass index; CRP, C-reactive protein; CVD, cardiovascular disease; Nt-Pro-BNP, N-terminal prohormone brain natriuretic peptide; PEW, protein-energy wasting.
[a]The low ghrelin group was defined as ghrelin values below the 33rd percentile (lower third) of distribution.
[b]Significantly different from the low ghrelin group if $P<0.05$, as assessed by Mann–Whitney U test or χ^2 test.
[c]Univariate correlation with ghrelin concentration as assessed by Spearman's rank test; asterisks denote statistical significance as follows: **$P<0.01$; ***$P<0.001$.
[d]Median value; 25th to 75th percentile shown in parentheses (all such values).
[e]Prevalence, shown in percentage (all such values).
[f]PEW was defined as Subjective Global Assessment >1.
[g]Average ± s.d. (all such values).

Table 2 | Clinical and biochemical characteristics in 217 hemodialysis patients, according to ghrelin and nutritional status[a]

	Not wasted (*n*=115)		Wasted (*n*=102)		
	High ghrelin (*n*=75)	Low ghrelin (*n*=40)	High ghrelin (*n*=70)	Low ghrelin (*n*=32)	MANOVA[b]
Ghrelin, pg/ml	423 (358–522)[c]	219.2 (138–253)	435 (382–619)	256 (212–279)	—
Age, years	64 (48–73)	66 (46–79)	62 (53–71)	75 (65–81)	G, W
Men, %	47[d]	80	46	50	0.004[e]
Vintage, months	28 (13–57)	34 (19–59)	29 (14–66)	22 (11–54)	NS
Diabetes, %	20	27.5	27.1	31.3	NS[e]
CVD, %	57.3	62.5	68.5	71.8	NS[e]
BMI, kg/m^2	24.9 ± 5.8[f]	25.7 ± 3.9	22.9 ± 4.5	25.6 ± 5.9	G
Serum albumin, g/l	36.5 ± 3.9	36.0 ± 3.3	33.2 ± 4.3	31.9 ± 5.1	W
Total cholesterol, mmol/l	4.5 ± 1.1	4.4 ± 1.0	4.2 ± 0.9	4.2 ± 1.2	NS
CRP, mg/l	4.1 (1.8–15.5)	5.2 (1.8–8.2)	10.0 (3.8–27.0)	12.4 (4.1–31)	W, G × W
Nt-Pro-BNP, pg/l	4.6 (2.2–12.9)	3.7 (1.7–17.1)	11.5 (5.2–3.5)	14.3 (3.2–3.5)	W
Adiponectin, μg/ml	22.2 (14.6–29.9)	17.2 (10.6–25.7)	26.9 (15.4–37.9)	23.5 (14.3–26.7)	G, W
Leptin, ng/ml	20.4 (6.0–61.4)	17.4 (7.8–44.7)	8.0 (3.6–22.5)	34.6 (8.9–91.3)	G × W
Leptin/BMI	0.78 (0.27–2.16)	0.74 (0.34–1.65)	0.37 (0.20–0.85)	1.25 (0.50–3.35)	G × W

Abbreviations: BMI, body mass index; CRP, C-reactive protein; CVD, cardiovascular disease; MANOVA, multivariable analysis of variance; NS, nonsignificant; Nt-Pro-BNP, N-terminal prohormone brain natriuretic peptide; PEW, protein-energy wasting.
[a]The low ghrelin group was defined as ghrelin values below the 33rd percentile (lower third) of distribution, whereas PEW was defined as Subjective Global Assessment >1.
[b]Two-factor MANOVA. Significant ($P<0.05$) effects are given for ghrelin (G), PEW (W), and the interaction ghrelin × PEW (G × W).
[c]Median value; 25th to 75th percentile shown in parentheses (all such values).
[d]Prevalence, shown in percentage (all such values).
[e]Assessed by χ^2 test.
[f]Average ± s.d. (all such values).

characteristics of the patients according to this categorization are detailed in Table 2. Patients with low ghrelin were older, had higher BMI, and presented lower adiponectin. Patients with PEW were also older and exhibited signs of inflammation (increased C-reactive protein (CRP) levels and lower serum albumin) and elevated N-terminal prohormone brain natriuretic peptide values. A significant ghrelin × PEW interaction was found for CRP and leptin values: patients with both PEW and low ghrelin values exhibited the highest CRP concentrations and the highest leptin values (Figure 1). The latter was true even after normalization by BMI, that is, leptin levels indexed to BMI.

Survival analysis for these four groups showed, as expected, a negative impact of PEW but also a detrimental impact for concurrent low ghrelin values. Thus, across the four ghrelin-PEW categories, the percentage of deaths during follow-up (both from all- and CVD-related causes) was incrementally higher (Table 3). In Kaplan–Meier curves, this group division resulted in a worse outcome because of both all-cause (log-rank (χ^2) 24.61; $P<0.0001$) and CVD-related (log-rank (χ^2) 15.55; $P=0.001$) mortality (Figure 2). Crude and adjusted Cox proportional HRs are depicted in Table 3, choosing as the reference the group without PEW and elevated ghrelin values. Regarding all-cause mortality, both wasted groups exhibited a worse outcome in crude and adjusted analysis, being considerably worse in magnitude for the group of wasted patients who also had low ghrelin. The HR of wasted patients with low ghrelin compared with wasted patients with high ghrelin was 2.05 (95% CI 1.17–3.57), a difference that persisted after multivariate adjustment (HR 1.96, 95% CI 1.11–3.50). Regarding CVD-related mortality, it was only the group of patients with both PEW and low ghrelin values who had worse outcome in both crude and adjusted models (Table 3). The HR for CVD

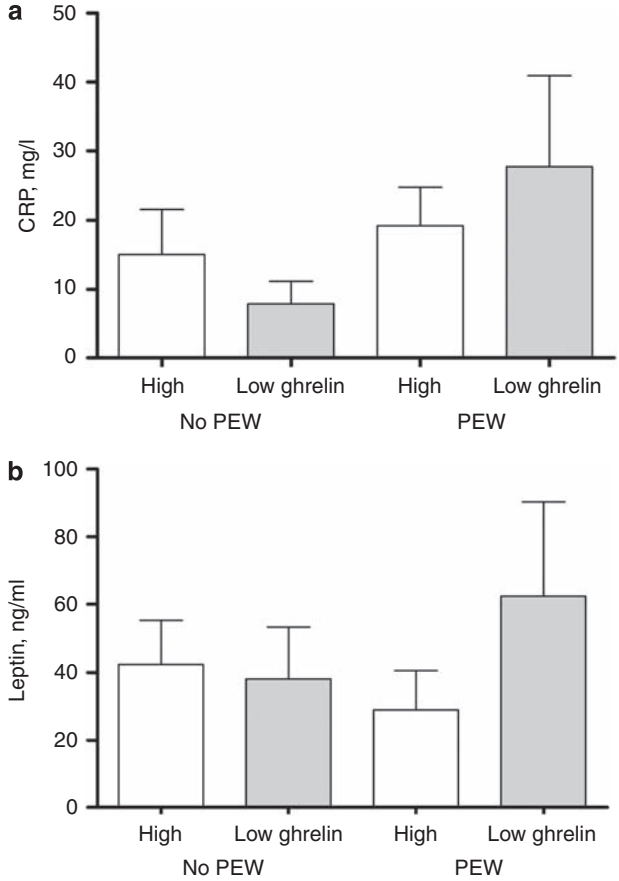

Figure 1 | PEW modifies the cross-sectional association of ghrelin with circulating CRP and leptin. Levels of (**a**) C-reactive protein (CRP) and (**b**) leptin among ghrelin and wasting categories cross-classified in 217 prevalent patients undergoing hemodialysis. Data are presented as average values with error bars depicting 95% confidence intervals. Multivariable analysis of variance (ANOVA) showed a significant ghrelin × wasting interaction for both CRP and leptin values. PEW, protein-energy wasting.

Table 3 | Crude and adjusted all-cause and CVD-related mortality according to ghrelin and wasting groups[a]

		Non-wasted, low ghrelin		Wasted, high ghrelin		Wasted, low ghrelin	
Model	Covariates	HR (95% CI)	*P*-value	HR (95% CI)	*P*-value	HR (95% CI)	*P*-value
All-cause mortality							
1	Crude	1.51 (0.72–3.06)	0.2	2.09 (1.17–3.74)	0.01	4.33 (2.30–8.16)	<0.0001
2	1+age, sex, and vintage[b]	1.55 (0.74–3.18)	0.2	2.37 (1.31–4.32)	0.004	3.53 (1.84–6.74)	0.0002
3	2+diabetes and CVD	1.37 (0.64–2.82)	0.4	2.24 (1.24–4.16)	0.007	3.34 (1.74–6.41)	0.0003
Cardiovascular-related mortality							
1	Crude	0.92 (0.25–2.85)	0.9	1.61 (0.68–3.94)	0.3	4.36 (1.79–10.86)	0.001
2	1+age, sex, and vintage[b]	0.94 (0.25–2.92)	0.9	1.77 (0.73–4.44)	0.2	3.88 (1.56–9.50)	0.003
3	2+diabetes and CVD	0.78 (0.21–2.44)	0.7	1.64 (0.68–4.08)	0.3	3.54 (1.40–8.91)	0.008

Abbreviations: CI, confidence interval; CVD, cardiovascular disease; HR, hazard ratio.
[a]Indicated are univariate and multivariate HRs and 95% CIs for all-cause and CVD mortality. The group of patients who were non-wasted (defined as Subjective Global Assessment >1) and had high ghrelin (grouping middle and high thirds of serum ghrelin together) was used as a reference.
[b]Age was categorized according to <45, 45–65, and >65 years, selecting the youngest group as the reference; dialysis vintage was dichotomized to the round approximation of the median value (2 years), using the shorter vintage as the reference category; female sex, the absence of diabetes, and of CVD were considered reference categories.

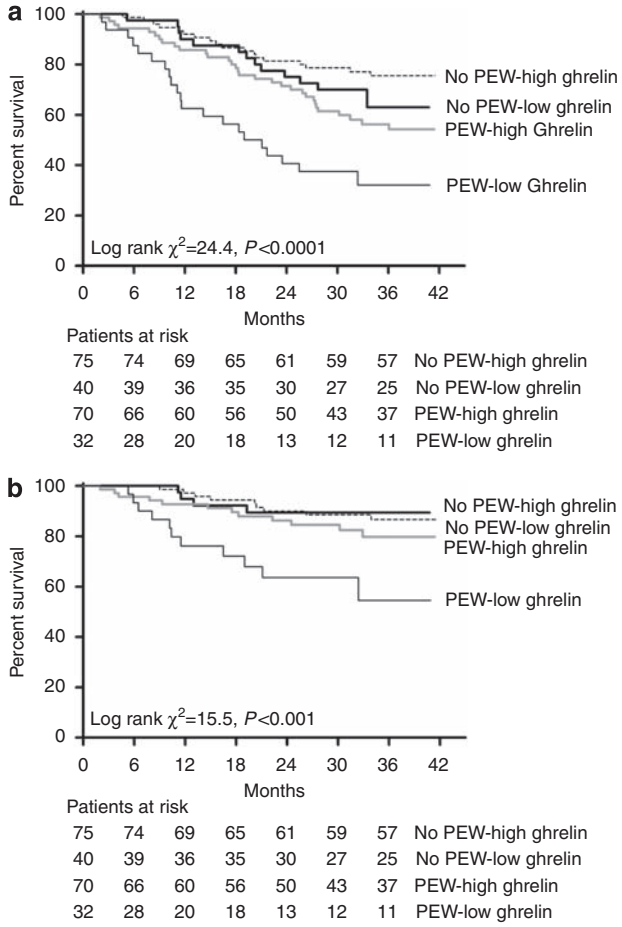

Figure 2 | PEW modifies the impact of low ghrelin values on mortality. Kaplan–Meier (**a**) all-cause and (**b**) cardiovascular-related mortality curves among ghrelin and wasting categories cross-classified in 217 prevalent patients undergoing hemodialysis. PEW, protein-energy wasting.

mortality of wasted patients with low ghrelin compared with wasted patients with high ghrelin was 2.63 (95% CI 1.15–5.99), a difference that persisted after multivariate adjustment (HR 2.78, 95% CI 1.18–6.52). As a sensitivity analysis, results were confirmed by excluding patients within the middle third of ghrelin, and comparing bottom vs top third only; HRs continued being statistically significant in both uni- and multi-variate models (data not shown). The causes of death are detailed in Table 4.

DISCUSSION

This study reports, for the first time in CKD and we believe in any patient group, an increased mortality risk for patients with low ghrelin values. Although this effect was modest and did not stand full adjustment for confounders, the prognostic value of low ghrelin on outcome seemed magnified in the context of PEW. Thus, wasted patients with low ghrelin concentration presented the highest mortality risk, especially cardiovascular-related mortality. This group of patients showed at the same time abnormally elevated serum CRP and leptin values, forming altogether a pattern of concomitant conditions that seem to fit with the purported anti-inflammatory,[19,20] cardioprotective,[16,17] and orexigenic[14] effects attributed to ghrelin administration. Our study therefore identifies a group of dialysis patients who could, at least in theory, benefit from ghrelin treatment.[13,14]

To the best of our knowledge, the only available study linking ghrelin with mortality comes from an animal study where ghrelin injection early after myocardial infarction prevented an increase in cardiac sympathetic tone and reduced mortality (survival rate 77% in the treated group vs 39% in the placebo).[31] Our study shows that in dialysis patients low ghrelin levels were associated with an increased mortality risk, but that this increased risk was lost after correction for cardiovascular comorbidities, suggesting that cardiovascular comorbidities may, at least in part, operate within the same causal pathway of ghrelin and mortality.[16,17] It is tempting to speculate that the uremic phenotype with markedly increased prevalence of anorexia, PEW, inflammation, and CVD may magnify these interactions. In agreement with previous studies indicating that ghrelin is influenced by

Table 4 | Individual causes of death according to ghrelin and nutritional status[a]

	Not wasted		Wasted	
	High ghrelin (*n*=75)	Low ghrelin (*n*=40)	High ghrelin (*n*=70)	Low ghrelin (*n*=32)
Cardiac arrest/sudden death	4	1	2	2
Myocardial infarction	4	—	3	6
Cerebrovascular accident	—	—	3	1
Other causes of cardiac death	1	3	3	2
Hemorrhage	1	—	1	—
Pulmonary edema	—	—	1	—
Malignancy	1	1	—	2
Infection/septicemia	1	3	7	2
ESRD treatment withdrawn	3	2	2	1
Patient refused further RRT	—	—	2	—
Cachexia	1	—	2	—
Other	—	—	2	1
Uncertain/not determined	2	3	3	4
All deaths[b], *n* (%)	18 (24%)	13 (32%)	31 (44%)	21 (65%)
CVD deaths[b], *n* (%)	9 (12%)	4 (10%)	11 (17%)	11 (35%)

Abbreviations: CVD, cardiovascular disease; ESRD, end-stage renal disease; RRT; renal replacement therapy.
[a]Indicated are the causes and number of deaths (*n*) in each category.
[b]Indicated are the number of deaths and percentage, expressed as a proportion of the total number of patients in the group. The proportion of deaths was incrementally higher across the group as assessed by χ^2 test (P=0.004 for all deaths and P=0.03 for CVD deaths).

the individual's nutritional status,[27–29] our results also indicate that the increased mortality risk associated with a state of PEW is further aggravated by low ghrelin values. Indeed, this group of individuals showed the highest mortality risk, especially because of cardiovascular-related causes, which agrees with the present understanding of ghrelin's implications in cardiovascular physiology. Available literature provides some clues on the plausible mechanisms linking ghrelin with cardiovascular disease: indeed, in experimental models ghrelin has been shown to inhibit cardiomyocyte cell death,[32] improve left ventricular function,[17] suppress cardiac sympathetic activity, decrease plasma norepinephrine, and prevent early left ventricular remodeling.[33] In humans, a correlation between ghrelin levels and blood pressure has been observed,[24] and infusion of a ghrelin agonist decreases blood pressure and heart rate[34] and recovers ventricular function.[16] In dialysis patients, ghrelin was found to be linked to coronary microvascular, endothelial,[35] and left ventricular[36] dysfunction. Interestingly, in the absence of PEW, low ghrelin did not exert any detrimental effect on outcome.

As expected, we found a negative correlation between leptin and ghrelin, because while ghrelin stimulates, leptin inhibits food intake, competing for the same hypothalamic targets. At a neuronal level, ghrelin-induced activation of neuropeptide Y neurons is suppressed by subsequent administration of leptin.[37] Ghrelin and leptin thus reciprocally regulate neuropeptide Y neurons, and the balance of ghrelin and leptin levels seems critical for energy homeostasis regulation. However, the precise nature of the interaction between ghrelin and leptin and the underlying signaling mechanisms in neuropeptide Y neurons are largely unknown. In CKD, clinical studies have yet not been able to demonstrate a role of hyperleptinemia in anorexia or PEW, and a state of leptin resistance has been proposed.[38] Because CRP may directly block the effects of leptin upon satiety and weight reduction,[39] systemic uremic inflammation may contribute to leptin resistance. It is plausible (although not yet demonstrated) that the regulation of ghrelin metabolism may add to this complex scenario. The wasted patients in our study with low ghrelin values presented abnormally high leptin concentrations (even after correction for BMI), providing a scenario with a clear balance shift toward a pro-anorectic situation. This group of patients was also the most inflamed and presented the worst outcome. Ghrelin administration has been shown to inhibit proinflammatory responses and nuclear factor-κB activation in human endothelial cells, monocytes, and T cells.[19,20] Also, in a rat model of CKD, 14 days of ghrelin infusion resulted in improved lean body mass and less inflammation.[21] In agreement with this, the concurrent presence of low ghrelin and PEW in our study significantly increased the levels of CRP, which is in turn a well-recognized mortality risk marker.[40]

Given the extensive history of negative randomized controlled trials in the dialysis population, it is exciting that two small randomized controlled trials with ghrelin infusion therapy show encouraging results in improving food intake in malnourished dialysis patients.[13,14] As ghrelin may not only reduce systemic inflammation[19,20] but also improve cardiac performance,[16,17] our study identifies a specific group of wasted, inflamed, and ghrelin-deficient patients at increased (cardiovascular) mortality risk who potentially could benefit from this therapy. We should bear in mind, however, that the evidence so far in CKD is based on small-size short-duration trials and further studies regarding safety, way of administration, and side effects are still needed before its use could be recommended.[41]

Several important limitations of this study should be noted, and taking these into consideration we believe that our

results are only hypothesis generating. First, because it is a cross-sectional design, the present analysis is limited in its ability to establish causal relationships. Second, as our cohort was relatively small and consisted of prevalent patients (a group of survivors), our findings need confirmation in larger as well as incident patient cohorts. Third, samples were taken in non-fasting conditions, 1–3 h after a meal. Although this may have affected ghrelin values, we should bear in mind that the parameters to which ghrelin is related to in this study (PEW, leptin, CRP, and outcome) are not affected by the postprandial state. Additionally, total ghrelin levels decrease very modestly after a standardized meal in dialysis patients, as opposed to healthy individuals.[42] What factors trigger postprandial ghrelin suppression are presently unknown, but postprandial suppression of ghrelin in healthy volunteers occurs independent from the previous day's intake and does not relate to subsequent food intake.[43] Also, a high-protein diet reduced food intake in healthy individuals despite compensatory changes in ghrelin concentration.[44] One more consideration is that because fatal cardiovascular events were extracted from patient records and not always confirmed by autopsies, the true prevalence of cardiac end points could not be established, being possibly higher. Finally, our analysis relies on total ghrelin concentration, not differentiating between the different ghrelin isoforms. It would have been desirable to measure acylated ghrelin in this study, but at the time of data collection, little was known about ghrelin isoforms, and samples were not pretreated with protease inhibitors. Finally, PEW was defined on the basis of SGA, which is a widely used nutritional assessment in dialysis patients but not the only one. Thus, we cannot exclude that results may differ when using other methodology to define PEW. Notwithstanding these limitations, the availability of extensive data with detailed characterization of a wide range of important risk factors and end points, including inflammatory biomarkers, comorbidities, and outcome, strengthens the study.

In conclusion, we report that low ghrelin values in wasted hemodialysis patients are linked to a markedly increased mortality risk, especially because of cardiovascular causes. As these patients exhibited a more anorectic phenotype (increased inflammation and serum leptin), our results provide a scenario where ghrelin therapies may be particularly useful. Whether ghrelin administration could directly, or indirectly through its beneficial metabolic effects, improve long-term outcomes in this patient population is a hypothesis that deserves further attention.

MATERIALS AND METHODS

Study population

The study was performed at five dialysis units in Stockholm, and one at the Uppsala Academic Hospital in Uppsala, Sweden. This is a *post hoc* analysis of data arising from a cross-sectional study originally aiming at investigating the variability of inflammatory markers in patients undergoing hemodialysis. The protocol has been previously described in more detail and patient recruitment took place between October 2003 through March 2004.[4] Out of the 224 prevalent patients included in the study and followed for assessment of overall and cardiovascular mortality, ghrelin data could only be measured in 217 patients because of lack of stored plasma for the missing seven patients. A single clinician, who extracted data pertaining to the underlying CKD, CVD history, and diabetes, reviewed each patient's medical chart. Survival was determined after a mean follow-up of 31 (20–38) months, with no loss of follow-up of any patient. Cardiovascular mortality was defined as death resulting from coronary heart disease, sudden death, stroke, or complicated peripheral vascular disease. The study protocol was approved by the ethics committee of Karolinska Institute at Huddinge University Hospital, Stockholm, Sweden, and informed consent was obtained from each patient.

Nutritional status

BMI was determined on a dialysis day. A translation into Swedish[45,46] of the SGA questionnaire[47] was used to evaluate the overall nutritional status. In the validation of SGA measurements in dialysis patients against total-body nitrogen level, Cooper *et al.*[48] showed that SGA differentiated malnourished patients from those with normal nutrition, but failed to reliably distinguish the degree of malnutrition. For that reason, we dichotomized the variable into presence (SGA >1) or absence (SGA 1) of wasting signs. SGA measurements are used in this study as a surrogate of PEW.

Laboratory analysis

Non-fasting blood samples were collected before the dialysis session. The plasma was separated within 30 min and samples were kept frozen at $-70\,^{\circ}C$ if not analyzed immediately. Concentrations of high-sensitivity CRP, serum albumin (bromcresol purple), and total cholesterol concentration were determined using routine methods at the Department of Laboratory Medicine, Karolinska University Hospital, Huddinge, Sweden. N-terminal prohormone brain natriuretic peptide was measured by an automated immulite analyzer (Siemens Healthcare Diagnostics, Los Angeles, CA). Both plasma leptin (Linco Research, St Charles, MO) and plasma total ghrelin (Phoenix Pharmaceuticals, Belmont, CA) were measured by radioimmunoassay methods, using commercial assays and according to the manufacturer's instructions.

Statistical analysis

Normally distributed variables were expressed as mean ± s.d., and non-normally distributed variables were expressed as median and interquartile range (25th–75th percentiles). Categorical data were presented as percentage values. Comparisons between two groups were assessed with Mann–Whitney or χ^2 tests. Spearman's rank correlation (ρ) was used to determine correlations of ghrelin with other variables. As there is no clinically established cutoff value for low ghrelin levels, the lower third of ghrelin distribution in our patient population was considered as a low concentration. A two-factor multivariable analysis of variance with Wilk's λ was used to measure the degree of correlation between the variables. The model included a test for the effect of order. The general linear model procedure with least-squares means was used to identify significant interactions between factors. The χ^2 test was used for categorical variables. Survival analyses used the Kaplan–Meier survival curve and the Cox proportional hazards model. The univariate and multivariate Cox regression analysis are presented as HR (95% CI). All statistical analyses were performed with SAS statistical software

(Version 9.2; SAS Institute, Cary, NC), with statistical significance set at the level of $P<0.05$.

DISCLOSURE

BL is affiliated with Baxter Healthcare. All the other authors declared no competing interests.

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