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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).