

Subcutaneous Ghrelin Enhances Acute Food Intake in Malnourished Patients Who Receive Maintenance Peritoneal Dialysis: A Randomized, Placebo-Controlled Trial

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Anorexia and malnutrition confer significant morbidity and mortality to patients with end-stage kidney disease but are resistant to therapy. The aim of this study was to determine whether subcutaneous administration of ghrelin, an appetite-stimulating gut hormone, could enhance food intake in patients who are receiving maintenance peritoneal dialysis and have evidence of malnutrition. The principal outcome measure was energy intake during a measured study meal. Secondary outcome measures were BP and heart rate and 3-d food intake after intervention. Nine peritoneal dialysis patients with mild to moderate malnutrition (mean serum albumin 28.6 ± 5.0 g/L, total cholesterol 4.4 ± 0.6 mmol/L, subjective global assessment score of 5.7 ± 1.7) were given subcutaneous ghrelin (3.6 nmol/kg) and saline placebo in a randomized, double-blind, crossover protocol. Administration of subcutaneous ghrelin significantly increased the group mean absolute energy intake, compared with placebo, during the study meal (690 ± 190 versus 440 ± 250 kcal; $P = 0.0062$). When expressed as proportional energy increase for each individual, ghrelin administration resulted in immediate doubling of energy intake (204 ± 120 versus 100%; $P = 0.0319$). Administration of ghrelin maintained a nonsignificant increase in energy intake over 24 h after intervention (2009 ± 669 versus 1579 ± 330 kcal) and was not followed by subsequent underswing (1790 ± 370 versus 1670 ± 530 and 1880 ± 390 versus 1830 ± 530 kcal on days 2 and 3, respectively). Ghrelin administration resulted in a significant fall in mean arterial BP ($P = 0.0030$ by ANOVA). There were no significant adverse events during the study. Subcutaneous ghrelin administration enhances short-term food intake in dialysis patients with mild to moderate malnutrition.

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End-stage kidney disease is a chronic condition associated with a high prevalence of nutritional dysfunction (1). This malnutrition is resistant to intervention (2) and is a major predictor of morbidity and mortality in patients who receive both peritoneal dialysis (PD) and hemodialysis (3,4). There is a linear correlation between body mass index (BMI) and survival in dialysis patients (5), to the extent that the usual association of increased mortality with obesity is reversed in patients who receive renal replacement therapy (6). Nutritional parameters that have been correlated independently with increased mortality and morbidity include low serum albumin (4,7,8), low serum cholesterol (7,9), and a low measured subjective global assessment score of nutrition (4,10).

Malnutrition in chronic kidney disease is multifactorial, but reduced protein and energy intake play an important role (1). Patients with kidney failure experience a complex anorexic

syndrome that is evident early in the course of the disease, before the requirement for dialysis (11), including patients with a GFR >50 ml/min per 1.73 m² (12). Reduced dietary energy and protein intake is common in patients who are on maintenance PD and hemodialysis (1), and inadequate dietary intake correlates with poor nutritional outcomes (13). The effects of diminished dietary energy intake in some PD patients may be offset by increased glucose absorption from peritoneal dialysate (14). Proposed mechanisms for anorexia include derangement of gut hormones, reduced gastric emptying, and the anorectic effects of cytokines and uremic toxins (1).

The hormone ghrelin, principally secreted from the stomach (15), is thought to function as an appetite enhancer (16). Ghrelin is also important in long-term body weight regulation, as circulating ghrelin levels are inversely correlated with energy balance (17) and chronic ghrelin administration results in weight gain in animal models (18). Ghrelin mediates its orexigenic action *via* the type 1a growth hormone secretagogue receptor (15). Ligation of this receptor also results in release of growth hormone, although the appetite-enhancing effect of ghrelin is independent of growth hormone (19). The activity of ghrelin is dependent on acylation of the third serine residue (20). Secreted acylated ghrelin exerts orexigenic effect but is rapidly converted in

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the circulation to deacylated ghrelin, which does not activate the type 1a growth hormone secretagogue receptor and is not known to exhibit orexigenic effect or endocrine activity (15,20).

Intravenous infusion of ghrelin stimulates appetite in healthy volunteers (21) and cancer patients (22). Therefore, we hypothesized that subcutaneously administered ghrelin could enhance acute food intake among PD patients with evidence of malnutrition and provide the potential for practical therapeutic intervention.

Materials and Methods

Patients

Malnourished patients, between the ages of 18 and 55 yr, were recruited from the PD unit of Hammersmith Hospitals NHS Trust, which forms part of the West London Renal Centre. Thirty-eight eligible patients were invited to participate; 11 were interviewed, and nine patients consented to trial participation. The trial was performed between January and July 2004. Patients were defined as malnourished when they demonstrated two of three qualifying criteria: Serum albumin <35 g/L by Bromocresol Purple (normal range 35 to 48) with normal C-reactive protein, serum cholesterol <4.5 mmol/L, or subjective global assessment (SGA) score $\leq 6/7$ (23). A modified seven-point SGA score (severe malnutrition 1 to 2; mild to moderate malnutrition 3 to 5; mild malnutrition to well nourished 6 to 7) was used (4). Patients with a history of diabetes or coronary or cerebrovascular disease were excluded to minimize risk for intercurrent events. Factors that could confound food intake analysis, such as psychologic food aversion, abdominal discomfort during PD exchanges, dialysis fills <1 L, and recent initiation of PD (<6 mo), were also exclusion criteria. The Riverside Medical Ethics Committee approved the protocol (reference number 3721). Participants gave written consent, and the study was performed in accordance with the Declaration of Helsinki.

Protocol

The study was performed as a randomized, double-blind, placebo-controlled, crossover protocol in a dedicated clinical trials unit at Char-

ing Cross Hospital. Before initiation, patients attended a sham study session to acclimatize to study conditions and to ensure palatability of the study meal, which was assessed using a nine-point hedonistic scale. On subsequent study visits, patients received an injection of 3.6 nmol/kg subcutaneous ghrelin or saline in random order, separated by at least 7 d. A subcutaneous dose of 3.6 nmol/kg ghrelin was chosen as the smallest effective dose from a dose-response pilot study performed in healthy volunteers (Dr. N. Neary and Dr. M. Druce, Department of Metabolic Medicine, Imperial College London, UK, personal communication, November 2003). An independent physician performed the randomization of injection sequence.

On the day before each study meal, patients refrained from alcohol and strenuous exercise, consumed a standard-calorie prefast meal, and then fasted overnight from 9 p.m. On each study day, patients received their injection at 11.30 a.m. and were provided with their selected meal in excess at 12:00 p.m. The patients were placed in isolation and requested to eat until they felt comfortably full. The study meal, of known caloric content, was weighed before and after *ad libitum* feeding to measure energy intake. Each individual received an identical meal at each study visit. The average caloric density of meals was 620 kcal/100 g. Water intake was also measured during this period. Throughout the study, participants were encouraged to relax by reading or watching films. Clocks were removed from the study room to limit the effect of meal anticipation on appetite sensation. Visual analogue scales, 100 mm in length, were recorded at baseline and subsequently every 30 min to evaluate subjective feelings of hunger, nausea, and meal palatability. BP and heart rate were measured and serial blood samples were taken for hormone assays at baseline and at 15, 30, 60, 75, and 90 min after injection. All blood samples were collected from an antecubital fossa cannula into lithium/heparin tubes (LIP Ltd., Cambridge, UK) that contained 2000 kallikrein inhibitor units of aprotinin (Trasylol; Bayer, Newbury, UK) and stored on ice. After centrifugation, plasma was separated immediately and stored at -20°C until analysis. Diaries of food consumption were recorded by each participant for 72 h after each study visit. Study participants received careful guidance regarding completion of their food diaries, and caloric intake was estimated by

Table 1. Study participants' demographic and nutritional parameters^a

Patient	Gender	Age (yr)	Diagnosis	PD Type	Duration (Mo)	Albumin (g/L)	Cholesterol (mmol/L)	SGA Score	Estimated Dry Weight (kg)	BMI (kg/m ²)	First Injection
1	M	48	Chronic glomerulonephritis	APD	120	34	4.1	6	90.6	30.1	saline
2	M	52	Hypertensive nephropathy	CAPD	12	25	4.0	6	73	25.0	ghrelin
3	F	53	IgA nephropathy	CAPD	42	33	3.6	6	67	26.5	ghrelin
4	F	39	Tubulointerstitial nephritis	CAPD	30	33	4.1	6	73.4	25.9	ghrelin
5	F	49	Adult polycystic kidney disease	APD	23	29	5.3	5	55.6	19.7	saline
6	M	45	Hereditary nephritis	APD	36	20	4.1	5	47.3	17.6	saline
7	F	55	Adult polycystic kidney disease	APD	30	26	4.8	5	48.2	21.1	saline
8	F	55	Adult polycystic kidney disease	CAPD	7	33	5.3	6	75.6	30.1	saline
9	M	52	IgA nephropathy	APD	84	24	4.1	6	76.4	26.1	ghrelin

^aPD, peritoneal dialysis; SGA, subjective global assessment; BMI, body mass index; APD, automated peritoneal dialysis; CAPD, continuous ambulatory peritoneal dialysis.

decoding diaries with Dietplan-5 (Forestfield Software, Horsham, West Sussex, UK) nutritional analysis software.

Materials

The synthesized human ghrelin (GSSFLSPEHQRVQQRKESKKPPA-KLQPR; Bachem St. Helens, UK) was sterile on culture after 7 d, and the limulus amoebocyte assay for pyrogen was negative (Associates of Cape Cod, Liverpool, UK). A 10-fold dose (36 nmol/kg) of ghrelin was administered to C57BL/6 mice for toxicity testing; behavioral observation and blinded histologic examination revealed no abnormalities. The orexigenic bioactivity of synthesized human ghrelin was confirmed by administration to a cohort of male Wistar rats. On each study day, a blinded physician prepared the injections by the addition of sterile water to freeze-dried vials of ghrelin or saline. The participants received 0.2 ml of the dissolved substance into their abdominal subcutaneous tissue with the use of a 27-gauge needle.

Hormone Measurements

Total ghrelin, insulin, and peptide YY were measured using established in-house RIA (24–26). All samples were assayed in duplicate and within one assay to eliminate interassay variation. The ghrelin assay cross-reacted fully with acylated and deacylated human ghrelin but did not cross-react with any other gastrointestinal or pancreatic peptide hormones. Briefly, antiserum (SC-10368) was obtained from Santa Cruz Biotechnology (Santa Cruz, CA) and used at a final dilution of 1:50,000. The ^{125}I ghrelin was prepared with Bolton & Hunter reagent (Amersham International, Little Chalfont, UK) and purified by RP-HPLC using a linear gradient from 10 to 40% acetonitrile and 0.05% TFA over 90 min. The specific activity of ghrelin label was 48 Bq/fmol. The assay was performed in total volume of 0.7 ml of 0.06 M phosphate buffer (pH 7.2) that contained 0.3% BSA and was incubated for 3 d at 4°C before separation of free and bound antibody by charcoal absorption. The ghrelin assay was able to detect changes of 8 pmol/L (95% confidence interval [CI]) with an intra-assay variation of 9.5%. The insulin assay was able to detect changes of 6 pmol/L (95% CI) with an intra-assay variation of 5.4%. The peptide YY assay was able to detect changes of 2 pmol/L (95% CI) with an intra-assay variation of 5.8%. A commercially available assay was used to measure plasma leptin (Linco Research, St. Charles, MO). Growth hormone was analyzed using an Advantage automated chemiluminescent immunoassay (Nichols Institute Diagnostics, San Juan Capistrano, CA), and glucose levels were measured by an Olympus AU640 clinical chemistry analyzer (Melville, NY).

Statistical Analyses

Blinded analysis of food diary data was performed using Dietplan-5 nutritional analysis software. Statistical comparisons between treatments for food intake, preprandial visual analogue scores, and hormone levels were made using nonparametric paired *t* test. Comparison of repeated measurements of BP and subjective measures of nausea and fatigue were performed by two-way ANOVA. Statistical analysis was performed with Prism software (version 3.0; GraphPad Software, San Diego, CA). All values are expressed as mean \pm SE.

Results

Five female and four male patients, mean age 49.8 ± 1.7 yr, agreed to participate in the study, and all nine completed the protocol. Demographic and nutritional parameters are displayed in Table 1. All patients were in a clinically stable condition. Mean serum albumin for the group was 28.6 ± 1.7 g/L, with mean total cholesterol of 4.4 ± 0.2 mmol/L and mean SGA

score of 5.7 ± 1.7 . Six participants fulfilled all qualifying criteria for malnutrition, and three individuals fulfilled two of three criteria. No patient had a serum albumin within the normal range, although all patients had a C-reactive protein level within the normal range. The mean BMI was 24.7 ± 1.5 kg/m².

The National Kidney Foundation's Dialysis Outcomes Quality Initiative (27) and the UK Renal Association's target creatinine clearance of ≥ 60 L/wk per 1.73 m² (28) was achieved by all patients. Mean duration of PD was 42.7 ± 12.2 mo, and no patient had experienced peritonitis within 6 mo of the trial. Throughout the study period, there were no changes in dialysis delivery between the study interventions for any patient. Spe-

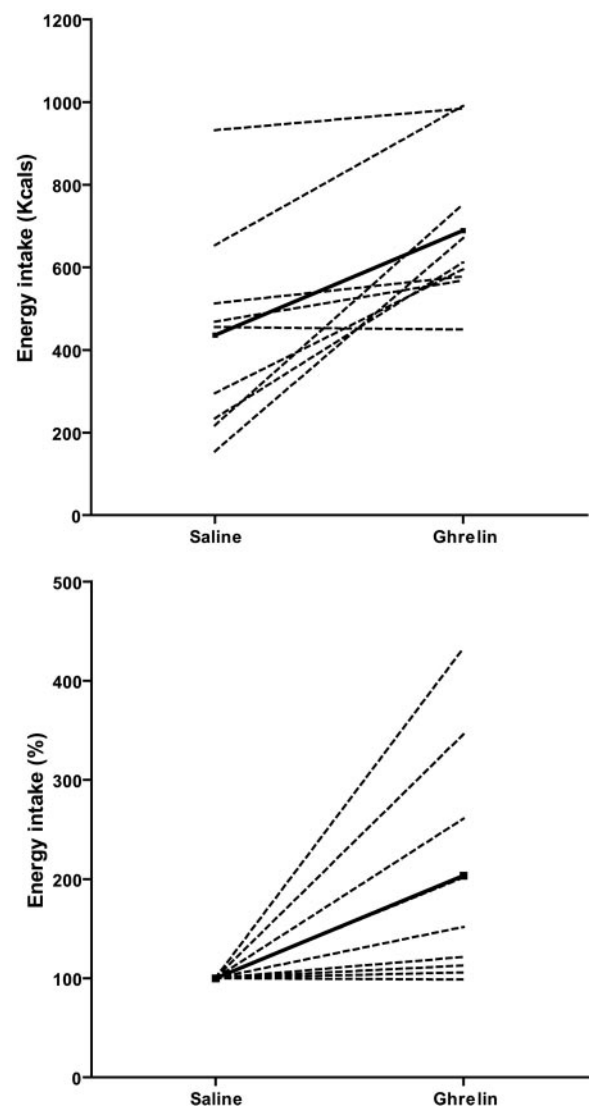


Figure 1. Energy intake after administration of subcutaneous saline or 3.6 nmol/kg ghrelin. (A) Absolute energy intake for individual participants during study meals, with mean absolute energy intake represented by the bold line. Data were analyzed by nonparametric paired *t* test. (B) Proportional increase in energy intake for each participant during study meals, with mean absolute energy intake represented by the bold line. Energy intake after saline injection was ascribed a value of 100%. Data were analyzed by nonparametric paired *t* test.

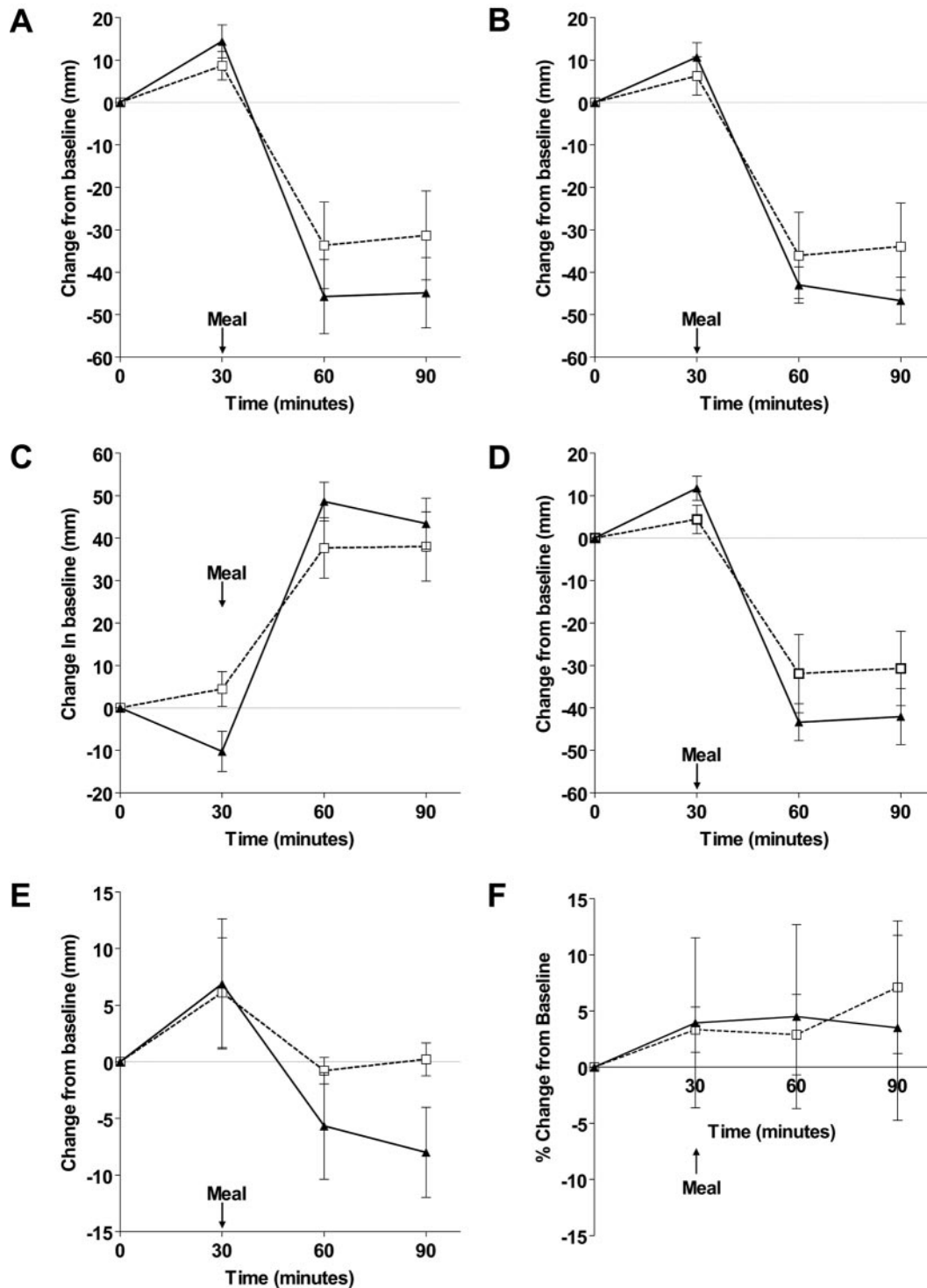


Figure 2. Subjective measures of appetite, nausea, and fatigue after administration of subcutaneous saline (dotted line) or 3.6 nmol/kg ghrelin (solid line). (A through F) Change from baseline values of visual analogue scores. Patients recorded their subjective assessment in response to a questionnaire asking (A) "How hungry do you feel?" (B) "How pleasant would it be to eat?" (C) "How full do you feel?" (D) How much do you think you can eat?" (E) "How nauseated do you feel?" (F) "How sleepy do you feel?" The injection was administered at 0 min, and the study meal was provided at 30 min.

cifically, each patient presented for his or her study meals with identical dialysate volume and glucose concentrations. There was no deviation from study timings, and each study partici-

pant received his or her meals at identical intervals after his or her last instillation of dialysis fluid.

Administration of subcutaneous ghrelin increased energy

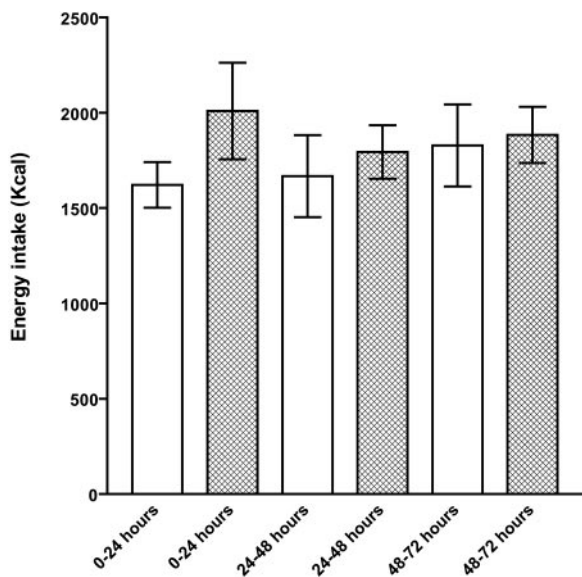


Figure 3. Energy intake calculated from diaries of food consumption, up to 72 h after administration of subcutaneous saline or 3.6 nmol/kg ghrelin.

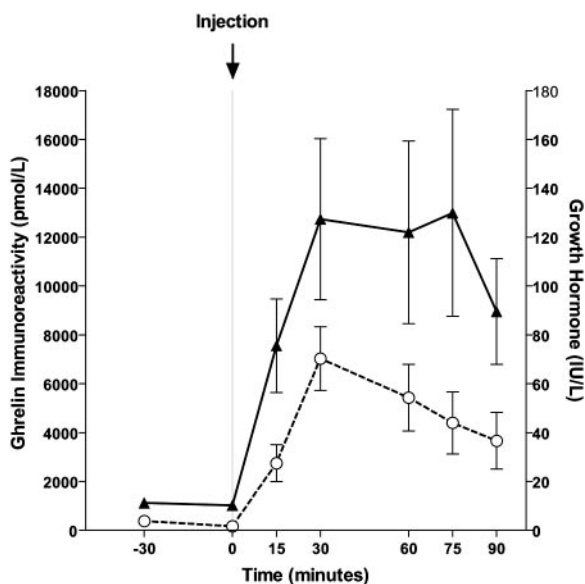


Figure 4. Plasma ghrelin immunoreactivity (solid line) and growth hormone levels (dotted line) after ghrelin administration. The injection was administered at 0 min, and the study meal was provided at 30 min.

intake during the study meal in eight of nine patients (Figure 1A). Significant differences in group mean absolute energy intake were observed between administration of subcutaneous saline and ghrelin (440 ± 250 versus 690 ± 190 kcal, respectively; $P = 0.0062$; Figure 1A). When expressed as proportional energy increase for each individual, ghrelin administration resulted in doubling of energy intake (100 versus $204 \pm 120\%$; $P = 0.0319$; Figure 1B). There were no significant differences in water intake after saline or ghrelin administration (132 ± 26 versus 165 ± 46 ml; $P = 0.4573$).

Visual analogue scores of subjective parameters of appetite were recorded before and after administration of the study injection (Figure 2). Administration of ghrelin was associated with changes in visual analogue scores, which were consistent with a subjective increase in preprandial appetite (Figure 2, A through D). However, in comparison with changes observed after saline administration, the observed differences in appetite scores after ghrelin administration did not achieve statistical significance for the subjective scores of hunger (saline 9 ± 3 mm versus ghrelin 14 ± 4 mm; $P = 0.2668$; Figure 2A), desire to eat (saline 6 ± 5 mm versus ghrelin 11 ± 4 mm; $P = 0.4905$; Figure 2B), fullness (saline 4 ± 4 mm versus ghrelin -10 ± 5 mm; $P = 0.0972$; Figure 2C), and expected food consumption (saline 4 ± 3 mm versus ghrelin 12 ± 3 mm; $P = 0.2014$; Figure 2D).

There was a nonsignificant trend for enhanced meal enjoyment after ghrelin administration when assessed by postprandial food palatability score (saline 57 ± 8 mm versus ghrelin 65 ± 7 mm; $P = 0.0921$). Throughout the study period, there were no significant differences in nausea ($P = 0.2171$ by ANOVA; Figure 2E) or fatigue ($P = 0.9294$ by ANOVA; Figure 2F) between saline and ghrelin administration.

Blinded analysis of food diaries, completed by the patients over the 3 d after injection, suggested that increased energy intake after ghrelin administration was maintained over 24 h, although this was nonsignificant (saline 1579 ± 130 kcal versus ghrelin 2009 ± 250 kcal; $P = 0.0645$; Figure 3). Importantly, the increase in energy intake after ghrelin administration was not followed by an underswing (saline 1670 ± 210 kcal versus ghrelin 1790 ± 140 kcal; saline 1830 ± 220 kcal versus ghrelin 1880 ± 150 kcal on days 2 and 3, respectively; Figure 3).

Patients with end-stage kidney disease demonstrated significantly higher circulating leptin levels (214.3 ± 4.6 pmol/L) than 16 BMI-matched healthy subjects (80.0 ± 2.0 ; $P < 0.0001$). Baseline hormonal analysis also revealed that study participants had significantly higher endogenous total plasma ghrelin immunoreactivity (1076 ± 92 pmol/L) than BMI-matched healthy control subjects (738 ± 104 pmol/L; $P = 0.0330$). After administration of subcutaneous ghrelin, the patients' total plasma ghrelin immunoreactivity increased, reached a plateau at 30 min (mean 12737 ± 3300 pmol/L; range 1421–25787 pmol/L), and started to fall after 75 min (Figure 4). As expected, growth hormone concentrations also increased from baseline (2.6 ± 0.6 IU/L) and peaked at 30 min (70.3 ± 13.0 IU/L), indicating that the administered exogenous ghrelin was bioactive (Figure 4).

Administration of ghrelin was not associated with changes in preprandial insulin levels (saline 86.2 ± 25.5 pmol/L versus ghrelin 82.3 ± 14.7 pmol/L; $P = 0.7957$), preprandial glucose concentration (saline 4.33 ± 0.09 mmol/L versus ghrelin 4.56 ± 0.12 mmol/L; $P = 0.2209$), or peptide YY levels (saline 54.4 ± 8.4 pmol/L versus ghrelin 51.3 ± 8.4 pmol/L; $P = 0.6571$) 30 min after injection. Administration of ghrelin was associated with a significant decline in mean arterial pressure from a baseline 110.4 ± 6.5 mmHg ($P = 0.0030$ by ANOVA; Figure 5A), diastolic BP from mean baseline 88.6 ± 4.2 mmHg ($P = 0.0039$ by ANOVA; Figure 5D), and a nonsignificant trend toward fall in systolic BP from mean baseline 132.1 ± 9.4

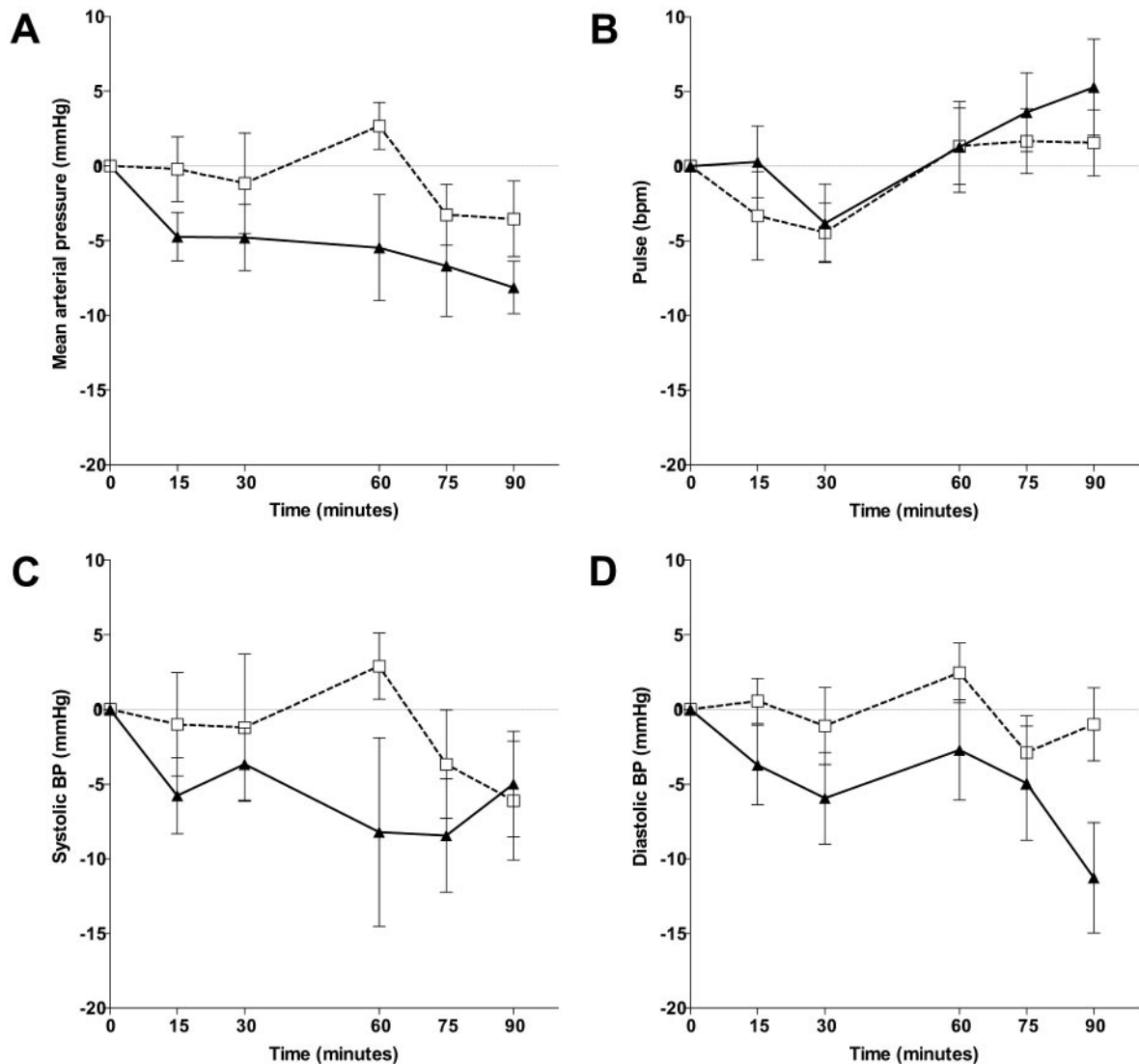


Figure 5. BP during the study meal, after administration of saline (dotted line) or 3.6 nmol/kg subcutaneous ghrelin (solid line). The change from participants' baseline mean arterial BP, pulse rate, and systolic and diastolic BP is shown in A, B, C, and D, respectively. All data were analyzed by a two-way ANOVA.

mmHg ($P = 0.0747$ by ANOVA; Figure 5C). The change in BP was evident within 15 min of ghrelin administration and was maintained for the 90-min observation period. No symptomatic hypotension occurred during the study. Administration of ghrelin was not associated with a change in pulse rate ($P = 0.2358$ by ANOVA; Figure 5B). There were no adverse events during the study period.

Discussion

Subcutaneous ghrelin administration resulted in a two-fold increase in short-term energy intake for each individual in a cohort of mildly to moderately malnourished PD patients. The mean absolute energy intake for the group increased from 440 ± 80 to 690 ± 60 kcal after a 10-fold increase in circulating ghrelin levels. The immediate increase in energy intake at the study meal was followed by a trend toward increased energy intake over the following 24 h (Figure 3). Importantly, there

was no subsequent compensatory reduction in energy intake over the following 72 h, which would negate any potential therapeutic benefit of chronic administration.

We demonstrated elevated endogenous total ghrelin levels in this cohort of PD patients, compared with BMI-matched healthy individuals, in agreement with observations by Perez-Fontan *et al.* (29) and Ayala *et al.* (30). Although there are high endogenous levels of total ghrelin, dietary energy intake is paradoxically reduced in PD patients. There are a number of possible explanations. First, this may reflect other abnormalities of appetite signaling, which are present in patients with kidney disease. For example, the current study also demonstrated elevated circulating leptin levels in PD patients, in keeping with previous observations (31,32), and inappropriately elevated leptin concentrations are associated with weight loss (33). Dialysis patients also display abnormally high concentrations of other anorectic gut peptides that may contribute to appetite loss and gastrointestinal dysfunction.

tion, including motilin, somatostatin, peptide YY, vasoactive intestinal peptide, pancreatic polypeptide (34), and cholecystokinin (35). Second, recent evidence suggests that the ratio of deacylated to acylated ghrelin is disordered in kidney disease and is a more potent driver of renal anorexia than total ghrelin immunoreactivity (36).

Despite the disordered endocrine system demonstrated in patients with kidney disease, this study demonstrates that the orexigenic effect of exogenous ghrelin administration is retained in patients with kidney disease and that there is no apparent ghrelin resistance associated with elevated total endogenous ghrelin levels. Indeed, we demonstrated a greater increase in food intake than previous trials of intravenously administered ghrelin to healthy individuals and anorexic cancer patients (21,22). This may reflect the high circulating total ghrelin levels achieved and subsequent correction of a disordered deacylated to acylated ghrelin ratio.

A significant fall in mean and diastolic BP was observed during study sessions, without symptomatic hypotension or reflex tachycardia. This effect seemed additive to the patients' ongoing antihypertensive therapy, which was not altered during the study; eight of nine patients were regularly taking antihypertensive medication, five of these requiring multiple therapy. A reduction in BP was seen throughout the study meal period and is in accordance with previous observations concerning the beneficial effects of intravenous ghrelin on BP and cardiac output in healthy volunteers and patients with heart failure (37,38). There is a close association between malnutrition and cardiovascular death in patients with end-stage kidney disease (39). In addition, emerging data also suggest an anti-inflammatory role for ghrelin (40). Ghrelin receptor ligation on T cells and monocytes inhibits the expression of proinflammatory anorectic cytokines such as IL-1, IL-6, and TNF- α and is hypothesized to provide a link between the metabolic axis and immune system (41). Epidemiologic data reveal an intimate relationship among inflammation, malnutrition, and cardiovascular outcomes (42,43). Thus, a therapy that is able to improve energy intake, reduce inflammation, and enhance cardiovascular function would be of great value to patients with kidney disease.

This study was designed as a short-term, single-dose analysis of the effects of ghrelin administration to establish efficacy. Patients with established cardiovascular disease were excluded to minimize adverse events; thus, few patients with severe wasting nutritional deficiency were eligible. However, it is these patients who are most likely to derive the greatest clinical benefit from treatment, and further investigation is needed to establish the efficacy of exogenous ghrelin within this high-risk group. Nonetheless, we have shown clearly that subcutaneous ghrelin administration is able to increase substantially acute spontaneous energy intake in mildly to moderately malnourished patients who receive maintenance PD. Long-term ghrelin administration may have the potential to improve nutritional parameters and patient outcomes. Longer term studies are now required.

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