Original Article

Increased plasma leptin/fat ratio in patients with chronic renal failure: a cause of malnutrition?

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Abstract

Background. Protein-energy malnutrition occurs in patients with chronic renal failure primarily due to loss of appetite. The *ob* gene protein, leptin, which is secreted by adipocytes, regulates body composition by lowering food intake. We have measured plasma leptin in undialysed and dialysed patients and in controls and the concentrations have been related to body composition, dietary intake, and biochemistry.
Methods. Plasma leptin was measured by radioimmunoassay in 93 individuals in groups of undialysed, peritoneal dialysed, and haemodialysed patients and controls. Body composition was determined by DEXA.
Results. Protein-energy malnutrition was evident in non-dialysed and dialysed patients from low lean or fat tissues, plasma albumin and transferrin. A third of the dialysis patients were eating less than prescribed intakes. Leptin relative to total fat mass (ng/ml/kg) was significantly greater for patients than for controls, particularly the dialysed patients. Leptin was highly correlated with total, arm, leg, and all other fat measurements, e.g. r for leptin vs% total fat was: undialysed 0.88, PD 0.81, HD 0.93, and controls 0.83 ($P < 0.0001$ for all). Dialysis patients with the highest leptin/fat mass ratio had low protein intakes and significantly lower lean tissue mass. Leptin/fat ratio correlated inversely with dietary intake e.g. with protein intake in g/day and marginally in g/kg of ideal weight/day. Leptin concentration was unrelated to plasma creatinine or residual renal function or to the protein 'nutritional indices', albumin and transferrin.
Conclusions. Our data suggests that leptin is markedly increased in some patients with chronic renal failure. The association of increased leptin with low protein intake and loss of lean tissue is consistent with leptin contributing to malnutrition but a definitive role cannot be substantiated by this study.

Key words: body composition; chronic renal failure; dialysis; dietary intake; fat; leptin

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Introduction

Protein-energy malnutrition occurs frequently in chronic renal failure (CRF) [1,2] and is associated with increases in morbidity and mortality [3,4]. Numerous factors contribute to malnutrition, including disturbances of protein and energy metabolism, hormonal imbalance, losses of amino acids and proteins into dialysis fluids, and particularly reduced food intake due to anorexia, nausea, and vomiting.

The obesity gene protein, now known as leptin, with a molecular weight of 16 kDa, was first cloned in 1994 [5]. Recent studies have shown that leptin has an endocrine function for lowering food intake and regulating body composition [6–8] and is produced exclusively in fat cells from animals and humans. In humans, leptin correlates with percentage body fat but obese persons are insensitive to this endogenous leptin production [9]. Leptin resistance in obesity is probably due to a reduced efficiency of brain leptin delivery [10,11].

A recent study in this unit investigated total and regional body composition in undialysed and dialysed patients with CRF and controls [12]. The patients had significant lean tissue depletion together with lower percentage of body fat than controls. The aim of this study was to measure plasma leptin in these subjects to establish whether leptin concentrations, or their relationship with body composition and dietary intake, could account for any loss of appetite or the incidence of malnutrition.

Subjects and methods

Ninety-three individuals were investigated: 11 female and 12 male undialysed CRF patients (UD) with serum urea > 30 mmol/l or creatinine > 500 µmol/l; 12 female and 12 male peritoneal dialysis (PD) patients including 21 undergoing standard continuous ambulatory peritoneal dialysis receiving three or four exchanges of 1.5–2.5 l of dialysis fluid per day and three receiving nocturnal intermittent peritoneal dialysis (10–20 l exchange without a daytime dwell); 11 female and 11 male haemodialysis patients (HD) receiving two or three sessions of 4-h dialysis per week, using bicarbon-

ate-buffered dialysate and cuprophane dialysers; 12 female and 12 male normal controls [12]. All subjects were Caucasian and free from acute illness within the previous 3 months, and none were receiving corticosteroids. Diabetic subjects were excluded.

Dietary protein intake, per kg of ideal body wt, was prescribed as follows: UD, 0.6–0.8 g; PD, 1.1–1.3 g; and HD, 1.1–1.2 g. All patients were prescribed 30–35 kcals/kg ideal body wt, depending on age, BMI, and the presence of malnutrition. Actual dietary intake in dialysis patients was measured by 3-day diet diaries supervised by the dietitian. Ideal body wt was derived using the mean body mass index (weight/height^2) of healthy individuals on an adequate diet. Blood samples were collected in EDTA tubes and immediately separated and stored at $-70°C$. Samples were non-fasting and collected in the morning before dialysis or at a comparable time for undialysed patients and controls. Leptin is unaffected by food ingestion [13,14].

Body composition was determined using DEXA as described previously [12] with a Lunar DPX-L scanner (Madison, WI, USA). Total and regional analyses were performed. Leptin was measured in plasma by radioimmunoassay (Linco Research, St Louis, MO) using a polyclonal antibody raised in rabbits against highly purified recombinant human leptin [13]. The detection and upper limits of the assay were 0.5–100 ng/ml, respectively. Samples above the upper limits were repeated in duplicate after dilution in buffer. The intra-assay coefficient of variation ranged 3.4–8.3% (i.e. on 10 replicates from five samples of varying concentrations) and the inter-assay coefficient of variation ranged 3.6–6.2% (i.e. between five separate assays of the above samples). Creatinine, bicarbonate, albumin, transferrin and cortisol were assayed by standard techniques. This study was approved by the hospital ethics committee and all subjects gave informed consent.

Statistics

Multiple two-sample Wilcoxon test was used for comparisons of patient groups with controls using a significance level of $(P \times m)$ where m was the number of comparisons made. All descriptive data are expressed as the mean and standard error (SEM) or median/range. Correlations between continuous variables were evaluated by Spearman correlation. Analysis of covariance was used to compare regression lines for two groups.

Results

Mean and SEM values for age, height, dry weight, BMI, total, arm and leg tissues, and other variables for the four groups are shown in Table 1. All groups were comparable in age and height but the haemodialysis patients had significantly lower body wt and BMI than the controls. Regional analyses by DEXA are more sensitive to lean tissue depletion than measurement of whole body tissue [12]. Whereas total lean and fat were not significantly different from controls, regional lean tissues were significantly different for haemodialysis patients (Table 1) and lower for some patients in the other groups. Arm fat was significantly lower in the undialysed CRF patients and arm and leg fat in the haemodialysis group. In addition to the decrease in lean tissue, dialysed patients, particularly those in the PD group, had low plasma albumin. Transferrin was low in all three groups. Plasma cortisol was increased marginally in the HD group and was higher than for the PD group ($P<0.001$).

Plasma leptin was higher in patients than controls and highly correlated with % total body fat in both groups (Figure 1). The regressions between leptin and % fat (or fat mass) for patients and control groups were significantly different ($P<0.001$). Concentrations of leptin were also higher in females than for males in both patients and controls but this could be attributed

Table 1. Mean ± standard error for patient characteristics, body composition, and other variables compared with controls. Dietry intakes for dialysis patients are also shown.

	Controls $n=24$	UD $n=23$	PD $n=24$	HD $n=22$
Age (years)	60.2 ± 1.6	54.9 ± 3.1	59.1 ± 3.1	61.0 ± 2.3
Height (cm)	165.0 ± 1.5	163.8 ± 1.8	163.4 ± 1.9	162.3 ± 1.8
Dry weight (kg)	72.4 ± 2.5	65.5 ± 2.7	65.8 ± 2.9	61.1 ± 2.7[a]
BMI (kg/m^2)	26.4 ± 0.7	24.4 ± 0.9	24.5 ± 0.8	23.0 ± 0.7[a]
Total fat (kg)	22.7 ± 1.9	16.9 ± 2.3	19.9 ± 1.7	17.2 ± 1.8
Arm fat (kg)	1.81 ± 0.13	1.22 ± 0.18[a]	1.55 ± 0.16	1.45 ± 0.17[a]
Leg fat (kg)	7.77 ± 0.82	5.89 ± 0.82	6.56 ± 0.65	5.13 ± 0.50[a]
Total lean (kg)	47.0 ± 1.7	46.5 ± 2.0	43.5 ± 1.9	41.68 ± 1.82
Arm lean (kg)	5.3 ± 0.2	4.8 ± 0.3	4.5 ± 0.3	4.3 ± 0.3[a]
Leg lean (kg)	15.7 ± 0.6	14.9 ± 0.7	14.0 ± 0.7	13.1 ± 0.7[a]
Albumin (g/l)	39.3 ± 0.5	36.9 ± 1.2	31.6 ± 0.9[c]	35.9 ± 0.6[c]
Transferrin (mg/dl)	270 ± 7.4	217 ± 7.8[c]	202 ± 6.1[c]	204 ± 8.1[c]
Bicarbonate (mmol/l)	26.1 ± 0.5	17.3 ± 0.7[c]	24.3 ± 0.5[a]	19.0 ± 0.7[c]
Creatinine (µmol/l)	95 ± 2.0	769 ± 43[c]	842 ± 50[c]	844 ± 38[c]
Cortisol (nmol/l)	321 ± 30	388 ± 35	270 ± 15	383 ± 24 $P=0.065$
Energy/ideal weight kcal/kg/day	No data	No data	28.7 ± 1.2	25.6 ± 1.4
Protein/ideal weight g/kg/day	No data	No data	1.00 ± 0.04	1.00 ± 0.06

[a]$P<0.05$.
[b]$P<0.01$.
[c]$P<0.001$.
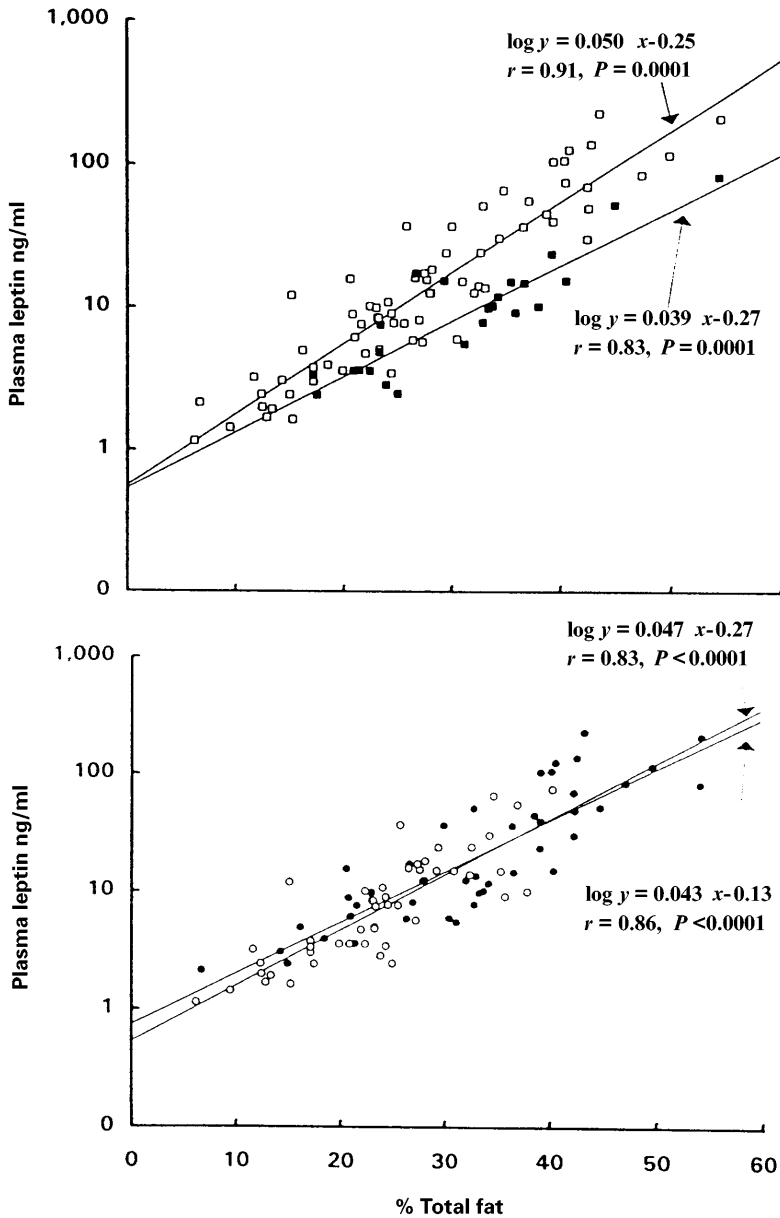
Fig. 1. Plasma leptin concentrations (ng/ml) are shown on a logarithmic scale against the percentage of total fat in patients ($\square$, $n=69$) and controls ($\blacksquare$, $n=24$). The regressions for patients and controls were significantly different. In the lower diagram all individuals are shown as males ($\bigcirc$, $n=47$) and females ($\bullet$, $n=46$).

to the higher percentage of fat in the females, as the regressions were similar for both groups (Figure 1). The regression line for plasma leptin against BMI in females was significantly different to that for males, because of their greater body fat content at a particular BMI [8]. Concentration of leptin was higher for patients in the PD group than for controls (Figure 2) although they had similar fat mass, consequently the leptin/fat mass ratio was markedly higher. Leptin was markedly increased for several individuals in the UD and HD groups although the increase was not significant. Fat was relatively lower than for controls and the increase in leptin/fat mass ratio was significant for the HD group.

Plasma leptin was highly correlated with total fat in men and women separately, and combined, e.g. r for leptin vs percentage total body fat measured by DEXA was for controls 0.83, UD 0.88, PD 0.81, and HD 0.93 ($P<0.0001$ for all), and similar r values were observed for leptin with total fat mass. As would be expected, there was an inverse correlation between leptin and percentage lean tissue, but leptin also correlated inversely with total lean tissue mass for the combined groups of patients ($r=-0.39$, $P<0.0001$), indicating that high leptin is associated with low lean tissue mass in chronic renal failure, particularly in patients with less than their ideal body wt. Plasma creatinine was significantly increased in all patient groups, and bicarbonate was lower than for controls although neither variables correlated with plasma leptin or leptin/fat
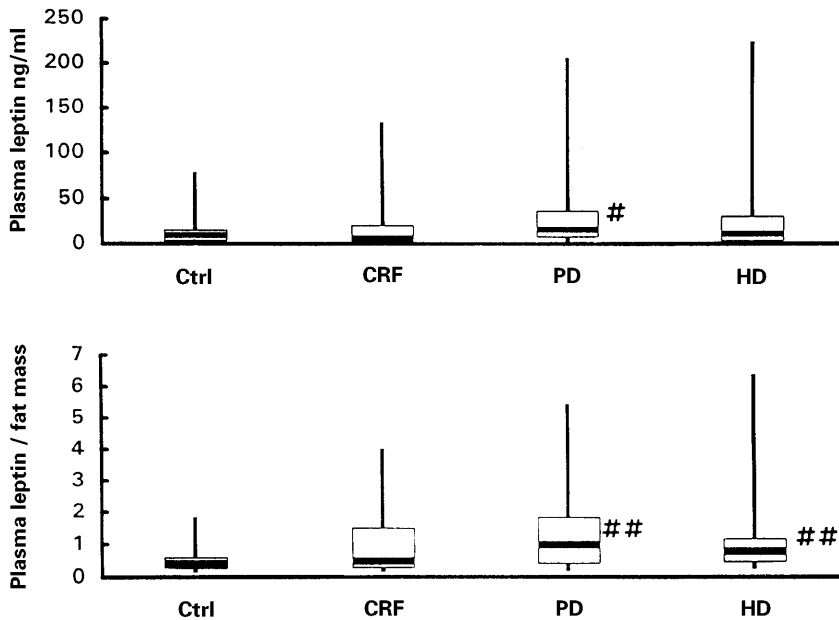
Fig. 2. Plasma leptin and the ratio of plasma leptin to total fat mass in controls and in patients who were undialysed (UD), peritoneal dialysed (PD) and haemodialysed (HD). Data are medians, 25th, 75th percentiles and ranges. Controls vs patient groups, *$P<0.05$, **$P<0.01$.

ratio for the individual or combined groups. However, creatinine did correlate weakly with leptin in females in the PD group and female controls ($r=0.58$, $P=0.05$ for each). No significant correlations were observed between leptin and residual renal function or K_T/V in dialysis patients, or between leptin and creatinine or urea clearances in UD or PD groups or between leptin and age, or plasma protein 'nutritional indices, i.e. albumin and transferrin, in UD, PD, or HD patients. Plasma cortisol correlated inversely with total lean tissue in each patient group and when combined ($r = -0.33$, $P=0.006$, $r=69$), but not with plasma proteins.

Actual dietary intake was monitored in the dialysis patients and protein was <0.9 g and calories <25 kcals/kg of ideal body wt, in over a third of the patients (seven receiving PD and 12 on HD). Mean calorie intake (including glucose absorbed from peritoneal dialysis fluid) (Table 1), was marginally higher in PD than HD patients ($P=0.07$). Protein, but not energy intake was related to leptin/total fat measurements (Figure 3). Also in the combined groups, total lean tissue mass correlated positively with dietary intake of protein and also calories, $r=0.44$, $P=0.003$ ($n=45$) for both. The 10 dialysis patients with the highest leptin/fat mass ratios were consuming low protein intakes and the mean intake (0.91 g/kg of ideal body wt) was marginally lower than for those with lower leptin (1.1 g, $n=35$, $P=0.065$). Mean total lean tissue for these 10 patients (37.3 kg, $n=10$) was also lower than for the others (44.1 kg, $n=35$, $P<0.05$). Plasma albumin, transferrin and residual renal function were not significantly different between the two groups.

Normal urine did not contain measurable amounts of leptin. Greater amounts were present in the urine of patients with chronic renal failure and this is being further investigated. After ultrafiltration of either urine or plasma from these patients, through a membrane filter of 10 000 Dalton molecular weight limit, only trace amounts of leptin were present in the filtrate. This suggests that degradation products less than 10 000 Dalton, do not contribute to measured leptin levels. However, this does not exclude the possibility that molecules larger than 10 000 Dalton might be present and cross react with the assay.

Discussion

Our results show that plasma leptin is closely related to adiposity in patients with chronic renal failure and in controls with normal renal function. The essential difference is that patients tend to have higher leptin despite lower body fat so that the ratio of leptin to fat is greater, particularly for patients receiving peritoneal or haemodialysis. Some patients had values for leptin higher than those previously reported for obese patients [15]. Higher values for females have been attributed to specific gender or hormonal effects [16] but the similarity of the regression lines for males and females in this study i.e. plasma leptin against fat (Figure 2), as opposed to BMI suggests that higher leptin could be due mainly to the higher percentage of body fat in the females.

Several features associated with chronic renal failure could contribute to increased production of leptin or decreased loss, and it is likely that both occur, although our data cannot differentiate between these effects. However, increased expression of leptin relative to fat mass may be stimulated by hyperinsulinaemia [15–17] or high cortisol [18], and both are common in renal
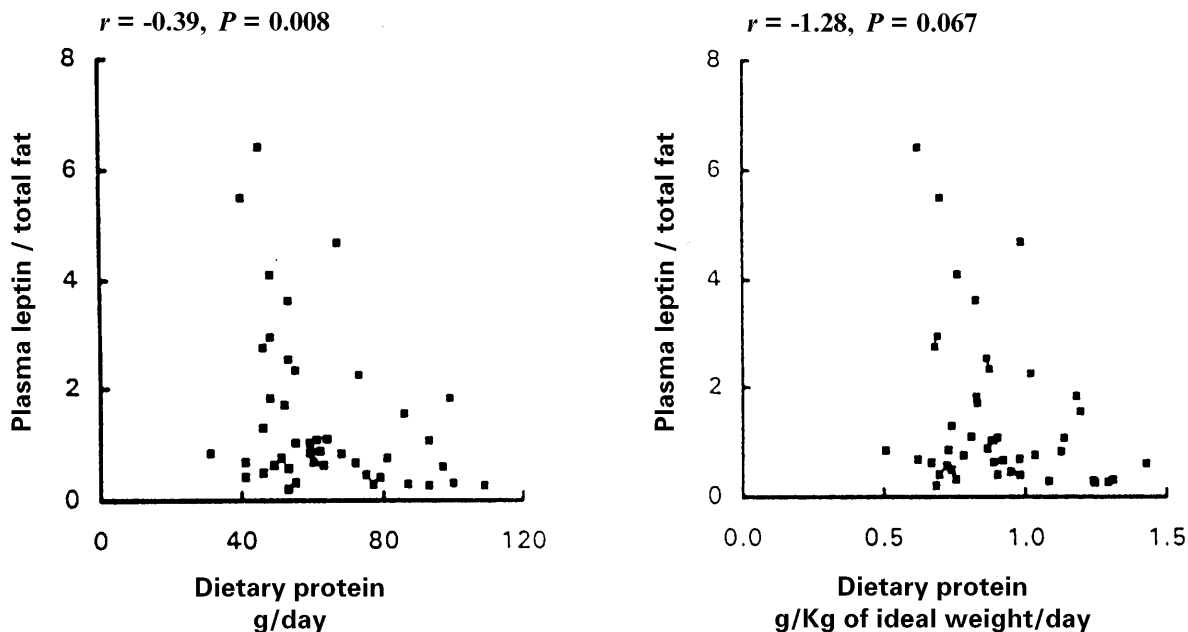
Fig. 3. Plasma leptin/total fat mass correlated with dietary protein intake expressed in g/day and marginally in g/kg of ideal body wt/day.

failure. Cortisol was measured and concentrations were higher for HD patients. Plasma creatinine, urea, or loss of residual renal function were generally unrelated to leptin, suggesting that uraemia or inadequate dialysis were not the main cause. However, lack of correlation between creatinine, as a marker of renal function, and leptin could also be due loss of lean tissue, the effects of dialysis, and in the non-dialysed group, to a narrow range of creatinine values. Increases of leptin could be caused by prolongation of the half-life due to decreased degradation. The kidney plays a major role in the catabolism of several hormonal proteins and recently the clearance of plasma leptin in rats has been shown to be attributable to the kidney [19]. In this study the absence of leptin in normal urine would be consistent with catabolism in the kidney. The presence of small but variable amounts of leptin in the urine of patients reflects the low molecular weight proteinuria seen in advanced renal disease.

Several studies have shown that leptin enters the brain by a saturable process independent of insulin [10] where it binds to receptors, principally in the hypothalamus and choroid plexus. One explanation for its effect on appetite is that it reduces the concentration of neuropeptide Y, a peptide that stimulates food intake. In lean subjects the majority of leptin circulates in a protein-bound form that may be unavailable to brain receptors for its inhibitory effects on food intake [20], whereas in obesity the majority of leptin is in the free, bioactive form, but a reduced efficiency of brain leptin delivery results in an apparent leptin resistance [11]. However, these authors also show that very high concentrations may overcome the resistance by allowing increased diffusion into the brain. In this study patients had high leptin concentrations relative to their fat mass which might cause increased uptake

by the brain in chronic renal failure, particularly if it is in the bioactive form. Further studies are necessary to establish whether leptin is present in the bioactive or protein-bound form or as a high molecular weight fragment. Our dialysis patients were eating less than prescribed amounts of protein, relative to ideal body weight, and they had low lean tissue mass. Consequently, the increased leptin-like immunoreactivity might be expected to be in the bioactive form if this is consistent with suppression of appetite for protein. Such an interpretation would imply that the appetites of patients with low protein intake, despite low plasma leptin, would be suppressed by other factors. However, such cause and effect relationships cannot be substantiated by this cross-sectional study. Leptin/fat ratio did not correlate with energy intake probably because of the glucose absorbed from the fluid in the PD patients, i.e. an involuntary and disproportionate increase in energy compared with that in HD patients.

Some degree of malnutrition was evident in non-dialysed and dialysed patients as suggested by low plasma albumin and transferrin, and lean or fat tissues. The HD group had significant loss of lean tissue and a higher cortisol concentration than the PD group who received a higher calorie intake and had lower plasma albumin. Similar differences were first observed between marasmic and kwashiorkor malnutrition and were attributed to the hormonal balance and calorie/protein ratio [21]. More than a third of the dialysis patients were eating less than prescribed intakes and they had lost body wt, lean tissue, and some of these patients, notably those on PD, had low albumin. Consequently, neither lean tissue or leptin correlated with the plasma protein 'indices of nutrition'. The associations between increased leptin and low intake

of dietary proteins, dietary intake and lean tissue mass, and also between low tissue mass and high leptin, may indicate that leptin is a cause of malnutrition, but we cannot exclude other common underlying factors which could increase leptin production, cause loss of lean body mass, and decrease dietary protein intake.

In summary, leptin in relation to body fat mass is greater in chronic renal failure than in individuals with normal renal function. The high concentrations found are associated with inadequate dietary intake and a corresponding depletion of lean tissue. Further studies are necessary to substantiate whether high leptin contributes to loss of appetite and the incidence of protein-energy malnutrition.

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