Cholecystokinin and leptin: their influence upon the eating behaviour and nutrient intake of dialysis patients

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Abstract

Background. We have used serial visual analogue scores to demonstrate disturbances of the appetite profile in dialysis patients. This is potentially important as dialysis patients are prone to malnutrition yet have a lower nutrient intake than controls. Appetite disturbance may be influenced by accumulation of appetite inhibitors such as leptin and cholecystokinin (CCK) in dialysis patients.

Methods. Fasting blood samples were drawn from 43 controls, 50 haemodialysis (HD) and 39 peritoneal dialysis (PD) patients to measure leptin and CCK. Hunger and fullness scores were derived from profiles compiled using hourly visual analogue scores. Nutrient intake was derived from 3 day dietary records.

Results. Fasting CCK was elevated for PD (6.73 ± 4.42 ng/l vs control 4.99 ± 2.23 ng/l, P < 0.05; vs HD 4.43 ± 2.15 ng/l, P < 0.01). Fasting CCK correlated with the variability of the hunger (r = 0.426, P = 0.01) and fullness (r = 0.52, P = 0.002) scores for PD. There was a notable relationship with the increase in fullness after lunch for PD (r = 0.455, P = 0.006). When well nourished PD patients were compared with their malnourished counterparts, CCK was higher in the malnourished group (P = 0.004). Leptin levels were higher for the dialysis patients than controls (HD and PD, P < 0.001) with pronounced hyperleptinaemia evident in some PD patients. Control leptin levels demonstrated correlation with fullness scores (e.g. peak fullness, r = 0.45, P = 0.007) but the dialysis patients did not. PD nutrient intake (energy and protein intake, r = −0.56, P < 0.0001) demonstrated significant negative correlation with leptin.

Conclusion. Increased CCK levels appear to influence fullness and hunger perception in PD patients and thus may contribute to malnutrition. Leptin does not

Keywords: appetite; cholecystokinin; dialysis; leptin; malnutrition; nutrient intake

Introduction

Malnutrition is common in dialysis patients and is associated with adverse outcomes. Numerous factors lead to depletion of body tissue and nutrients in ESRF. In order to compensate for this the predicted dietary requirements for dialysis patients are greater than for healthy subjects. Dietary intake surveys indicate that dialysis populations fail to achieve these targets. The discrepancy between high nutrient requirements and decreased nutrient intake suggests that there is disruption of the appetite regulation systems. Appetite regulation can be regarded as a series of feedback loops governing satiation (the process which stops ingestion at the end of a meal), satiety (the process which provides background inhibition of feeding urges) and feeding (which occurs in the absence of satiety) [1]. A considerable number of circulating molecules and neural pathways have been implicated in these pathways as well as various psychosocial factors.

Hylander et al. [2] found that dialysis patients, especially those on PD, consumed less test meal than controls and had a reduced rate of food consumption. This led to the hypothesis that premature satiation occurred in dialysis patients, possibly as a consequence of delayed gastric emptying [3,4]. Various upper intestinal hormones have been identified as early satiation factors. One such group of hormones are the cholecystokinin (CCK). These are released in response to eating and induce a state of satiety via peripheral and central receptors [5,6]. CCK is metabolized by
the kidney [7], consequently levels can be raised in renal failure [8–10]. Given that CCK accumulates in ESRF and retards gastric emptying [5], it may be partly responsible for the premature satiation effect, although evidence to support this theory is lacking.

Other circulating hormones are believed to influence nutrient intake. One such hormone is leptin which inhibits nutrient intake and increases energy expenditure in rodent models. It has been associated with weight loss when administered to some obese humans [11]. Several studies have demonstrated hyperleptinaemia in patients with renal failure, especially those on peritoneal dialysis (PD) [12–14]. The significance of uraemic hyperleptinaemia remains unclear. Some studies have suggested that leptin may exert a weak effect upon nutrient intake [13,14] whilst others have not [12]. We have previously reported studies of appetite profiles in haemodialysis (HD) and PD populations [15,16]. We used an electronic appetite rating system (EARS) to collect hourly visual analogue scores of hunger and fullness from dialysis patients and controls. We used these to compile profiles illustrating how motivation to eat varied through the day and to derive summary statistics to compare the groups. We found that the HD profiles were similar to controls on the interdialytic day but markedly abnormal after dialysis with lower hunger and higher fullness scores. The PD profiles were also markedly abnormal with the usual pre-meal increase in hunger and decrease in fullness absent (Figures 1 and 2). Both dialysis groups consumed less nutrients than controls but had similar mean hunger and fullness scores suggesting that they had reset their appetite to a lower level of nutrient intake.

This study was designed to examine the relationship between fasting levels of CCK and leptin and the nutritional status, nutrient intake and derived appetite ratings of dialysis patients and healthy controls.

Subjects and methods

The hospital ethics committee approved the study and informed consent was obtained from all subjects. No individual with a history of surgery to the upper intestine was recruited. Forty-three healthy controls, 50 HD patients and 39 PD patients were recruited. All patients were clinically stable and had been established on outpatient treatment for at least 3 months. The aetiology of renal failure for the HD group was unknown for 12, chronic glomerulonephritis for 10, polycystic kidney disease for 8, ischaemic nephropathy for 8, acute glomerulonephritis for 3, atherosclerotic renal artery stenosis for 2 and bilateral renal infarction, acute tubular necrosis, diabetes mellitus, reflux nephropathy, cast nephropathy, myeloma and cystinosis for 1 each. All HD patients were on thrice weekly bicarbonate-based dialysis employing low-flux ‘biocompatible’ membranes (Asahi AM Bio-wet; Asahi Medical Co. Ltd, Tokyo, Japan; or Fresenius F-6 or F-8; Fresenius Medical Care, St Wendel, Germany). The mean Kt/V for the group was 1.20 ± 0.27.

The aetiology of renal failure for the PD group was unknown for 13, chronic glomerulonephritis for 10, ischaemic nephropathy for 4, acute glomerulonephritis for 2, polycystic kidney disease for 2, diabetic nephropathy for 2, reflux nephropathy for 2, interstitial nephritis for 1, myeloma for 1, hereditary amyloidosis for 1 and atherosclerotic renal artery stenosis for 1. Eighteen used CAPD and 21 used automated PD (APD) with a daytime dwell. Fifteen of the APD patients used icodextrin-based fluid for their daytime dwell. Two of the CAPD patients used icodextrin-based fluid for their night-time dwell. The mean weekly Kt/V for the group was 2.32 ± 0.45. The mean weekly creatinine clearance was 69.34 ± 24.57. Further demographic data are shown in Table 1.

All participants attended the dialysis unit in the morning having fasted since the preceding midnight. Fasting blood samples were drawn; then participants were instructed how to use the EARS and fill in dietary records. Anthropometric assessments were performed to produce the data shown in Table 1. Percentage body fat (%BF) was derived from four-site skinfold thickness measurements. The fat mass (FM) and lean body mass (LBW) were derived from the bodyweight and %BF. A subjective global assessment (SGA) was also used to rate participants as well nourished (A), mildly-moderately malnourished (B) or severely malnourished (C). Kt/V and protein catabolic rate (nPCR) were calculated for all groups from blood samples and urine collections [1]. The PD patients collected their effluent dialysis fluid for 24 h prior to the sampling point.

EARS recordings

The EARS utilized a palmtop computer (Psion series 3; Psion computers Ltd, London, UK) programmed with EARS software. The device was programmed to alarm at hourly intervals. Each time the alarm rang a series of questions appeared on the screen in succession. The results from two questions are reported here: ‘How hungry do you feel?’ and ‘How full do you feel?’. Each question was accompanied by a line with ‘extremely’ and ‘not at all’ displayed at either end. Participants used one of two keys to move a cursor left or right along the line thus indicating the strength of each feeling at each time point. Hourly scores were used to produce a profile between 08:00 and 20:00 for the controls and PD. HD entered data between 10:00 and 20:00 on the day of dialysis but between 08:00 and 20:00 on the interdialytic day. Both days of the HD recording were used for analysis. For a more detailed account of the methodology see Wright et al. [15].

The data were used to derive summary statistics. The mean score was produced from the individual’s average score for the day. The peak score was simply the highest score of the day. Both days of the HD recording were used for analysis. The SD scores indicate the standard deviation of each patient’s responses during each day to give an idea of the variability of the parameters tested.

Nutrient intake measurements

Three day diet diaries were used to assess protein, energy and water intake. Standardized advice was given and one
specialist renal dietician assessed all records. Total nutrient intakes were estimated using Microndiet 8.08 software (University of Salford, Manchester, UK). The total intakes for each day were averaged. As PD patients can absorb an appreciable amount of glucose from their dialysis fluid, the caloric value of the absorbed glucose was calculated and used to produce the ‘total energy intake’ data. This was calculated from the difference between the glucose content of the infused dialysate and that of the drained dialysate.

**Assay techniques**

Standard biochemical and haematological tests were performed in the hospital laboratory. The albumin assay used a bromocresol green technique. EDTA samples were collected for leptin and CCK assays. These samples were cooled before being spun, split into aliquots and frozen at −20°C whilst awaiting analysis. Leptin was measured using an ELISA technique (DSL-10-23100 kit; Diagnostic Systems Laboratories Inc., Webster, TX, USA). The manufacturers report an intra-assay CV of 1.5–6.2% and inter-assay CV of 3.3–5.3%. Plasma CCK was measured by a competitive radioimmuno-assay (Belfast Link Labs). The intra-assay CV was 13.3% at 5 ng/l and 11.5% at 15 ng/l. Interassay CV was 8.2% at 5 ng/l and 6.6% at 15 ng/l.

**Statistical analyses**

The leptin results were not normally distributed so a logarithmic transformation was applied. As leptin correlated with weight and FM, further analyses were performed on FM-adjusted data, although the untransformed figures are also provided for comparison. The appetite regulators underwent within-group analysis to compare the male and female levels with each other and post-hoc analyses. Between-group analyses used ANOVA, with Bonferroni-corrected t-tests used for post-hoc analyses. The groups were divided by SGA status to compare the derived EARS scores, nutrient intake data and the levels of the appetite regulators using Pearson’s method. The correlation analyses used nutrient intake values that had not been adjusted for body weight in order to prevent mathematical coupling with the FM-adjusted leptin levels. As a large number of correlation analyses were being performed, there was a high probability of generating a $P < 0.05$ by chance. For this reason, those analyses that achieved a significance level of $P < 0.05$ are reported in the Results, but the Discussion concentrates on those that returned a $P < 0.01$.

**Results**

The demographic data from the study groups are shown in Table 1. The dialysis groups had an excess of men compared with the controls. This was not statistically significant. The HD group was significantly older than the others ($P < 0.001$). None of the subsequent HD data correlated with age. When the PD group was divided according to modality (i.e. APD or CAPD), the APD group were noted to be younger than those on CAPD ($P < 0.0001$; PD vs control and HD). $P < 0.001$ vs control + PD; $P < 0.05$ vs control; $P < 0.001$ vs control.

Figures 1 and 2 show the mean hunger and fullness scores for the three groups. It can be seen that the PD group had the most abnormal profiles and the lowest nutrient intake (Table 1). Table 2 shows the levels of CCK and log([leptin]/kg fat) for the groups.

**Cholecystokinin**

CCK levels were not related to age, gender, body weight or FM so the unadjusted levels were used for analysis. PD levels were significantly higher than both control ($P < 0.05$) and HD ($P < 0.01$) (Table 2 and Figure 3). When the groups were divided according to SGA, there was no difference in CCK levels for control or HD but the malnourished PD patients had higher fasting CCK levels than the well nourished SGA controls ($P < 0.01$; HD, PD vs control and HD). When the PD group was divided according to treatment modality, the CAPD patients had higher fasting CCK levels than the APD patients (CAPD 8.5 ± 3.8 vs APD 5.4 ± 4.5 ng/ml, $P = 0.042$), reflecting the greater number of malnourished patients on CAPD.

**Leptin**

All three groups showed strong correlation between log([leptin]) and FM (control $r = 0.70$, HD $r = 0.81$, PD $r = 0.79$, all $P < 0.0001$) so the data are presented as log([leptin]/kg fat). Figure 5 illustrates that females had higher log([leptin]/kg fat) than males for all groups (control, $P < 0.001$; HD, $P = 0.004$; PD, $P < 0.001$). Between-group ANOVA indicated that there was significant variation for the male participants ($P < 0.001$). The male controls had significantly lower levels than

<table>
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<tr>
<th>Table 1. Demographic data for the study groups</th>
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<tr>
<td>Number</td>
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<tr>
<td>Sex (M/F)</td>
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<tr>
<td>Age (years)</td>
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<tr>
<td>Months on dialysis</td>
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<td>Weight (kg)</td>
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<td>BMI (kg/m²)</td>
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<tr>
<td>MAC (cm)</td>
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<td>%BF (%)</td>
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<td>FM (kg)</td>
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<td>Lean mass (kg)</td>
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<td>SGA</td>
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<tr>
<td>Protein intake</td>
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<td>Energy intake</td>
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Values represent mean ± SD except age and months on dialysis (median and range). Protein intake is shown as g/kg/day and energy intake as kcal/kg/day. When glucose absorption from the PD fluid was included, the PD energy intake was 28.4 ± 8.9 kcal/kg/day ($P = $NS vs control and HD). $P < 0.001$ vs control + PD; $P < 0.05$ vs control; $P < 0.001$ vs control.
both PD ($P < 0.01$) and HD ($P < 0.001$). The female participants also returned a positive ANOVA ($P < 0.001$). Control levels were significantly lower than both HD ($P < 0.01$) and PD ($P < 0.001$). PD females had significantly higher levels than HD ($P < 0.01$). When the groups were divided by SGA, there were no significant differences detected, nor were the leptin levels of the APD and CAPD patients different.

**Correlation analyses**

The controls and HD demonstrated little relationship between their EARS scores and CCK levels (Table 3). In contrast, the PD group had numerous positive relationships. Strong relationships with hunger and fullness SD and AFL were demonstrated. There was no relationship between nutrient intake and fasting CCK for any group.

The control group demonstrated correlation between log([leptin]/kg fat) and several EARS parameters, notably those derived from fullness scores. Neither HD nor PD demonstrated relationships between leptin and the EARS data. The energy intake of the control group was weakly correlated with the fasting leptin levels, whilst the HD group had no such relationship with nutrient intake. In contrast, the PD group demonstrated several negative relationships.
Fig. 3. Fasting CCK levels for the study groups. Bars indicate group means. PD mean was significantly higher than control ($P < 0.05$) and HD ($P < 0.01$).

Fig. 4. Fasting CCK levels according to SGA. There were no malnourished (SGA rating B/C) controls. Well nourished (SGA rating A) and malnourished HD patients had similar CCK levels. The malnourished PD patients had significantly higher levels than the well nourished ($P = 0.004$).

Fig. 5. Mean log(Leptin/kg fat) by gender and group. Bars indicate group means. Female levels were significantly higher than male levels for all groups. Results of between-group analyses are detailed in the text.
There was also a negative correlation between nPCR and log([leptin]/kg fat) \((r = -0.49, P = 0.002)\).

**Discussion**

It was notable that the PD group had the most abnormal appetite profiles, the lowest nutrient intake and the highest levels of CCK and leptin. These discrepancies between HD and PD suggest that the modality of dialysis may influence appetite. Given that the highest fasting CCK levels were found in the malnourished PD patients, we should focus our attention on this molecule as a potential target for future study and development of therapeutic agents.

Whilst the appetite profiles of dialysis patients were abnormal, the average values were not so different to controls suggesting that dialysis patients enter similar hunger and fullness values. Thus, if a patient has a high fasting CCK level every day, they will probably enter a similar baseline score to a control with a low fasting CCK level every day, they will probably enter a similar baseline score to a control with a low fasting CCK level every day, thus generating a more potent effect via both central and splanchnic pathways. CCK is usually metabolized by the kidneys. Residual renal function did not seem to influence the fasting CCK levels in the PD group as those producing <100 ml of urine per day had lower levels than those with residual renal function (5.6 ± 4.2 ng/l, vs 8.2 ± 4.5 ng/l, \(P = NS\)). When the kidneys fail, the function of peptide catabolism is often taken over by increased activity in other redundant catabolic pathways, frequently in the liver. It may be that PD interferes with these pathways whilst HD does not.

CCK administration causes premature satiation, reduced nutrient intake and an abbreviation of the eating period \([6,8]\). This matches the pattern described by Hylander et al. \([2]\) for her PD patients. CCK-8 also fits with some features of the 'uraemic toxin' postulated to suppress appetite in animal models \([17,18]\). It is within the 1–5 kDa fraction and exerts its anorectic effects via both central and splanchnic pathways. CCK is just one of several intestinal peptides that are present in high levels in ESRF patients \([19]\). It is plausible that other such molecules may also contribute similar effects.

The results of the leptin assays were in keeping with previous findings. Females consistently returned higher levels than males and the cross-group comparisons

### Table 3. Results of correlation analyses between fasting appetite regulators, derived EARS data and nutrient intake

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<th>Control</th>
<th>HD</th>
<th>PD</th>
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<tr>
<td></td>
<td>(r)</td>
<td>(P)</td>
<td>(r)</td>
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<tr>
<td>CCK Peak H</td>
<td>0.10</td>
<td>NS</td>
<td>-0.08 NS</td>
</tr>
<tr>
<td>SDH</td>
<td>0.07</td>
<td>NS</td>
<td>-0.07 NS</td>
</tr>
<tr>
<td>ΔFL</td>
<td>0.13</td>
<td>NS</td>
<td>-0.12 NS</td>
</tr>
<tr>
<td>Peak F</td>
<td>0.07</td>
<td>NS</td>
<td>-0.31 0.038</td>
</tr>
<tr>
<td>ΔHE</td>
<td>-0.36 0.028</td>
<td>NS</td>
<td>-0.05 NS</td>
</tr>
<tr>
<td>SDH ΔHE</td>
<td>0.39</td>
<td>0.020</td>
<td>0.46 0.005</td>
</tr>
<tr>
<td>Leptin Peak H</td>
<td>0.39</td>
<td>0.020</td>
<td>0.46 0.005</td>
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<tr>
<td>ΔHE</td>
<td>0.07</td>
<td>NS</td>
<td>0.10 NS</td>
</tr>
<tr>
<td>SDH ΔHE</td>
<td>0.52</td>
<td>0.001</td>
<td>0.12 NS</td>
</tr>
<tr>
<td>Energy Energy</td>
<td>-0.32</td>
<td>0.048</td>
<td>-0.01 NS</td>
</tr>
<tr>
<td>Protein Protein</td>
<td>-0.17</td>
<td>NS</td>
<td>-0.04 NS</td>
</tr>
</tbody>
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Parameters returning no correlation with a \(P < 0.05\) for any group have been omitted for clarity. Leptin results indicate correlation with log([leptin]/kg fat). H, hunger; F, fullness; NC, no correlation with \(P < 0.05\).
confirmed hyperleptinaemia for the dialysis groups [12–14]. The PD females were particularly prone to pronounced hyperleptinaemia. This is believed to be a metabolic consequence of PD.

The control group demonstrated several relationships between leptin and derived fullness scores. This may reflect leptin acting to promote a sense of satiety. Neither dialysis group demonstrated any significant relationship between EARS scores and leptin levels. This suggests that any effect leptin exerts upon appetite perception is masked in dialysis patients.

There is strong evidence that leptin regulates food intake in rodents but far less for humans. In dialysis patients, Johansen et al. [14] reported a negative correlation between nPCR and leptin levels. Our group previously reported a weak but statistically significant relationship between leptin:fat ratio and daily protein intake [13]. These relationships were the only evidence for an anorectic action of leptin in adults with renal failure. It is noticeable that the studies documenting pronounced hyperleptinaemia in PD patients have not assessed nutrient intake. Our data indicated highly significant negative relationships between log [leptin] / kg fat and nutrient intake for this group. Under-reporting may explain the relationship with the dietary parameters, but it cannot explain the relationship with nPCR suggesting that these correlations represent a true leptin effect.

It should be noted that CCK and leptin are not the only molecules implicated in appetite control. Bombesin, enterostatin, GLP-1, insulin, glucagon and somatostatin are a selection of potential peripheral appetite regulators. There are an even greater number of molecules implicated in the central regulatory pathways. In addition to this, the situation is made more complex by the effect of social and cultural interactions on eating behaviour. Nevertheless, it seems that both CCK and leptin may have important effects upon the ingestive process of PD patients. CCK may influence perceived fullness and contribute to the phenomenon of premature satiation, probably in association with other upper intestinal peptides. Leptin apparently has little effect on perceived appetite in the dialysis groups, but may well reduce the nutrient intake of some PD patients by its subtle central actions.

There is evidence that CCK and leptin interact. Matson et al. [20] injected mice with various combinations of intraperitoneal leptin, CCK and saline. They found that the combination of leptin and CCK reduced the caloric intake more than leptin alone. Isolated CCK administration did not reduce food intake but did reduce feeding during the post-injection period (unlike leptin alone). The reduced food intake brought about by the combined injections outlasted the circulating half-life of both peptides, implying a central synergism in this mouse model. The scenario described by this animal model is similar to the situation in some PD patients with high levels of both leptin and CCK. This may explain why the findings were different for the HD and PD groups. We suggest that this area requires further investigation to look for novel approaches to improve the nutritional status of malnourished PD patients.

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Conflict of interest statement. None declared.

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