Hemodialysis affects wanting and spontaneous intake of protein-rich foods in chronic kidney disease patients

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Running head: *Wanting* and intake of protein after hemodialysis.

Abbreviations: AA, amino acid; CKD, chronic kidney disease; HD patients, hemodialysis patients; nPCR, normalized Protein Catabolic Rate; PEW, protein energy wasting; TFEQ, three-factor eating questionnaire.

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ABSTRACT

Background: Protein-energy wasting is a risk factor for mortality and morbidity in hemodialysis patients (HD patients). Food intake could be modified by hemodialysis-related changes in the food reward system (*i.e.*, *liking* and *wanting* of specific macronutrients). In HD patients on days with and without dialysis, we evaluated: 1) the reward system for protein-, fat- and carbohydrate-rich foods, plasma hormones and metabolite changes; 2) the spontaneous *ad libitum* intake of macronutrients.

Methods: Twenty-four HD patients evaluated their *liking* and *wanting* of macronutrients at 7:30 am and 11:30 am on a day with and a day without dialysis. Concentrations of hormones and plasma amino acids were determined. An additional 18 HD patients ate what they wanted from a buffet lunch comprising eight dishes on a day with and a day without dialysis. Healthy subjects, age-, sex- and BMI-matched, served as controls.

Results: At 11:30 am, *wanting* for protein-rich foods was higher on the day with than on the day without dialysis (P<0.01), bringing *wanting* levels close to those of healthy subjects. This increase correlated with changes in the concentrations of plasma amino acids (P<0.01). HD patients ate more protein from the buffet on the day with than on the day without dialysis (P<0.01) and more than healthy subjects (P<0.01).

Conclusions: In HD patients, *wanting* and spontaneous intake of protein-rich foods increase immediately after dialysis. This increase correlated with decreased concentrations of plasma amino acids. Thus, in clinical practice, protein-rich foods should be recommended during and after dialysis in patients with protein-energy wasting.

Keywords: Reward system, food intake, feeding behavior, chronic kidney disease, haemodialysis

Abbreviations: AA, amino acid; CKD, chronic kidney disease; HD patients, hemodialysis patients; nPCR, normalized Protein Catabolic Rate; PEW, protein energy wasting; TFEQ, three-factor eating questionnaire.

INTRODUCTION

Hemodialysis patients (HD patients) have a high risk of malnutrition and protein energy wasting (PEW) (1). Around 30-50% of chronic kidney disease (CKD) patients suffer from PEW and approximately 10% of patients on chronic dialysis show signs of severe PEW (2, 3). PEW increases the risk of cardiovascular complications, infection and death (4-6). Many factors are responsible for malnutrition in HD patients (7-9). Possible causes of malnutrition include difficulty absorbing certain macronutrients and needing to regulate the intake of salt and the consumption of certain minerals and liquids. There are also issues with vitamin supplementation, irregular food intake, the dialysis procedure itself, gastrointestinal upset and changes in the organs involved in nutrient metabolism, variations in the levels of some appetite-regulating factors (*e.g.,* leptin), depression, anorexia, comorbidities, and the conditions that lead to CKD themselves. Alterations in taste and smell (7, 10) also contribute to the development of malnutrition.

Food intake is controlled by complex interactions between homeostatic, cognitive, and hedonic processes (11-13). Homeostatic processes induce feelings of hunger or satiety (14), and are mainly related to metabolic and neuroendocrine factors (12, 15). Cognitive control comprises self-control, imposed regimen, socioeconomic and cultural influences, environmental and emotional factors, and beliefs about food (11). Finally, hedonic control relies mainly on the reward system (16) which comprises three main components: "*liking*" (pleasure/palatability); "*wanting*" (appetite/incentive motivation); and "*learning*" (preference/aversion), which is produced by associative conditioning and cognitive processes (17, 18).

It is accepted that HD patients often experience decreased hunger (*i.e.,* impaired homeostatic control of food intake) (19-22) and that the cognitive control of food intake is significantly affected by stringent medical constraints (23, 24). It is also common knowledge that patients undergoing hemodialysis experience changes in food preferences, mainly in favour of sweet and salty foods, red meat refusal, and marked attraction to sour and strong flavors in general (25-27). However, to our knowledge, *liking* and *wanting* for the three types of macronutrients have never been studied in HD patients.

We therefore investigated the influence of hemodialysis on the hedonic control of food intake. In the first experiment, hunger, *liking* and *wanting* for the three macronutrients were evaluated before and after a normal scheduled session of hemodialysis. At the same time, plasma concentrations of hormones and amino acids (AAs) were measured. In the second experiment, spontaneous food choices and consumption of the three macronutrients were evaluated during a buffet lunch served after the hemodialysis. In both experiments, the same parameters were also measured in the same patients on a day without dialysis and in healthy subjects.

SUBJECTS AND METHODS

In EXP1, hunger, *liking* and *wanting* for the three macronutrients were evaluated in 24 HD patients before (7:00-8:00 am) and after (11:00 am-12:00 pm) their dialysis, and at the same times on a day without dialysis. These items were also evaluated in 24 healthy subjects matched for sex, age and body mass index (BMI). Blood samples were drawn from HD patients immediately before and after dialysis and at the same times, in healthy subjects.

In EXP2, the spontaneous choices and consumption of eight nutritionally varied dishes from a buffet lunch were evaluated in 18 others HD patients on a day with and a day without dialysis. Results were compared with those of 18-matched healthy subjects evaluated using the same protocol for two successive days.

HD patients had 3-morning dialysis sessions per week. In both experiments, the day of dialysis was randomly drawn within the week. HD patients (aged 18 to 80 years) had to be clinically stable for the previous preceding month. Healthy subjects had to have normal creatinine clearance (>60 mL/min). Exclusion criteria were diabetes, malnutrition (BMI<17.5 kg/m^2, transthyretin<0.25 g/L), congestive heart failure, acute or chronic infection, ongoing antibiotic treatment, active cancer or liver cirrhosis, smoking (>5 cigarettes/day), alcohol consumption (>3 units of alcohol/day), oral nutritional supplements, aversions to the foods used in the study.

For HD patients, additional data were recorded: type of kidney disease, length of hemodialysis treatment, type of dialysis membrane, persistence of residual diuresis, adequacy of the dialysis according to the KT/V (28), body weight 3 and 6 months prior the study, normalized Protein Catabolic Rate (nPCR) (28), medical history and adjuvant treatments.

EXP1

Study design

Before the experimental sessions, each participant completed the Three-Factor Eating Questionnaire (TFEQ) (29) to assess their food-related behavior. The TFEQ is a 51-item auto-questionnaire, which assesses three factors: the cognitive dietary restraint (conscious control of food intake with concerns about body shape and weight); the disinhibition of control (overconsumption of food due to a variety of stimuli associated with a loss of control over food intake); and the susceptibility to hunger (food intake or eating in response to feelings and subjective perceptions of hunger). All participants also completed a 3-day food eating questionnaire by recording all foods and beverages consumed during 3 days (one dialysis day, one day without dialysis and one weekend day), using household measures (a bowl, cup, glass, etc) to aid in portion size estimation.

On the morning of the sessions, HD patients and healthy subjects had to have their habitual breakfast (same composition and quantity before each session) at ~6:00 am (*i.e.* ~1.5

hours before the experimental measurements). Afterwards, participants could not eat until the end of the measurements. At ~7:30 am, in a neutral and calm environment, participants rated their hunger and evaluated their *liking* for six foods. The first two foods were rich in protein [Bündnerfleisch (dried beef) and imitation crab me], the next two were rich fat (melted butter and mayonnaise) and the last two were rich in carbohydrates (honey and strawberry jam). There were also two nonfood items (dishwashing liquid and toothpaste) used as controls. Each food and nonfood item were presented at random in separate small cups and smelled orthonasally for approximately 5-10 sec at a distance of 5-10 cm from the nose. Then, *wanting* for 18 other foods, presented as pictures showing typical protein-, fat- and carbohydrate-rich foods, was evaluated. The pictures showed, 1) a fried egg, grilled salmon, baked chicken, veal steak, rib steak, and turkey breast, 2) an avocado, olives, peanuts, chocolate, a doughnut, and whipped cream, 3) potato puree, rice, lentils, an apple, pasta, fruit cake. The pictures were presented separately for 10-15 sec in a random order. At ~11:30 am, participants rated again their hunger, *liking* and *wanting* using the same protocol. BMI was then evaluated and a dietician checked the 3-day dietary survey. During the interview, portion sizes were verified using the su.vi.max photos (30). Energy content and macronutrients composition of the ingested foods and beverages, were estimated using the nutrient software program Nutrilog Nutrition Sofware (Nutrilog SAS, France, 2016).

Subjective measurements

Participants indicated their hunger sensation on 10-cm visual analog scale (VAS) anchored at its ends by the statements "not at all hungry" and "very hungry". They also indicated on 10-cm VASs their *liking* and *wanting* with the following questions, respectively: “How much do you like the odor of this food now?”, “How much do you want to eat this food now?”

Blood parameters

Hemoglobin and plasma sodium, potassium, calcium, phosphorus, albumin, transthyretin (biomarker of PEW in HD patients), protein, blood urea nitrogen, creatinine, C-reactive protein, ghrelin (hunger hormone), leptin (satiety hormone), insulin, and AAs were determined. Plasmatic concentrations of ghrelin and leptin were measured with ELISA kits (Millipore, Billerica, USA for ghrelin; R&D systems, Abingdon, UK for leptin). Insulin levels were determined by chemiluminescent immunoassay (Immulite 2000 WPi, Siemens Healthcare GmbH, Erlangen, Germany). Plasma AA concentrations were evaluated after deproteinization with sulfosalicylic acid (Sigma-Aldrich, Saint Quentin Fallavier, France) by ion-exchange chromatography (Hitachi High-Speed Amino Acid Analyzer L-8900, Hitachi High-Technologies Corporation, Tokyo, Japan) as previously reported (31). In HD patients, parathyroid hormone, transferrin saturation and total plasma CO_2 were also measured.

EXP2

Study design

On the morning of the sessions, HD patients and healthy subjects were in the same feeding conditions as in EXP1. At ~12:30 pm (i.e. around their usual lunchtime), all participants rated their hunger and then evaluated their *liking* for eight dishes prepared by a caterer (see Supplemental Material 1) presented together on a tray. Each dish was smelled orthonasally then, each subject could choose the order and the quantities of each dish he wanted to eat. No time limit was imposed. The participants were not informed of the objectives of the study or that their intakes were precisely weighed. The entire meal contained 7417 kJ (1773 kcal) with 23% of energy from protein, 65% from fat and 12% from carbohydrates. At the end of the meal, hunger and *liking* were evaluated again for the eight dishes, and seven questions were added to evaluate how pleasant the meal was and the reasons for the subjects' food choices.

Subjective measurements

Hunger and *liking* for each dish were evaluated as in EXP1. Questions asked at the end of the meal were as follows: 1) Did you find the presented foods pleasant? 2) Were the presented foods varied enough? 3) Did you have enough to eat? Participants were also asked if their food choices were made: 4) According to the pleasure expected? 5) According to the dietary recommendations associated with your illness? 6) According to the nutritional value that you think you needed? and 7) According to your state of hunger? Responses were evaluated using 10-cm VASs with the statement "very unpleasant/too little variety/not at all" and "very pleasant/very diversified/yes, absolutely".

Blood parameters

All participants provided a blood sample (during the first session between 8:30 am and 9:30 am) to measure plasma albumin, transthyretin, urea, creatinine and C-reactive protein.

STATISTICAL ANALYSES

Values are expressed as the means ± SDs. Delta values indicate the difference in a parameter between the early (7:30-8:00 am) and late morning (11:30 am-12:00 pm). Power sample calculation was determined, in EXP1 from the expected change in *wanting* according to our previous study (32) and in EXP2, from the mean change in *wanting* of protein-rich foods in EXP1. Differences between the two experimental sessions for the HD patients were analyzed with two-way repeated measure ANOVAs (factor A: days; factor B: times of measurement) and with one-way repeated measure ANOVAs for healthy subjects. Values for the HD patients (days with or without dialysis) and those for the healthy subjects at a given time point were compared using one- or two-way ANOVAs (factor A: days; factor B: times of measurement). Whenever differences were significant, the Tukey *post hoc* test was applied. Anthropometric values, blood parameters and delta values were compared with Student's t-tests or the Mann-Whitney U-test. Spearman's correlation tests were used to screen for links between data. SigmaStat software (version 3.1, Systat Software Inc., Richmond, CA, USA) was used for analyses. Pearson's principal component analysis (PCA), with a correlation threshold at $P<0.02$, was done using XLSTAT software (version 2015, Addinsoft Inc., New York, USA). Statistical significance was set at $P< 0.05$.

RESULTS

EXP1

Participants' characteristics (Table 1)

There were no significant differences between HD patients and healthy subjects for sex, age and BMI. Participants' mean body weight was stable during the six months preceding the study.

In HD patients, compared to healthy subjects: hemoglobin levels were lower; albumin, protein, calcium and potassium concentrations in plasma were similar and; transthyretin, C reactive protein and serum phosphorus were higher.

Additional individual characteristics of HD patients are provided in Supplemental Material 2.

Attitude towards food and usual food intake (Table 2)

HD patients were more restricted and less disinhibited than healthy subjects. They were also less hungry overall.

Usual daily energy intake and fat intake did not differ between groups, whereas protein and carbohydrate intakes were lower in HD patients than in healthy subjects.

Hunger, liking and wanting

Hunger was more intense at 11:30 am than at 7:30 am in all participants (**Figure 1**). At 7:30 am, HD patients had similar levels of hunger on days with and without dialysis, but hunger was greater in HD patients than in healthy subjects on the dialysis day. At 11:30 am, hunger was similar in all participants.

Liking for protein-rich foods increased from 7:30 am to 11:30 am in HD patients and in healthy subjects (**Figure 2A**), whereas *liking* for fat-rich foods increased only in healthy subjects (**Figure 2B**) and *liking* for carbohydrate-rich foods did not change (**Figure 2C**). *Liking* for the three types of nutrients did not differ significantly between the participants at 7:30 am and at 11:30 am.

Wanting increased from 7:30 am to 11:30 whatever the participants' condition and the type of macronutrient (**Figure 3ABC**). At 7:30 am, there were no significant differences between HD patients and healthy subjects whatever the macronutrient. By contrast, at 11:30 am, *wanting* for protein-rich foods was lower on the day without than on the day with dialysis, and was lower than *wanting* reported by hemodialysis (**Figure 3A**). Consequently, *wanting* for protein at 11:30 am was similar in HD patients after dialysis and in healthy subjects. HD patients exhibited similar levels of *wanting* for fat and carbohydrate at 11:30 am whether they underwent dialysis or not (**Figure 3BC**). Finally, at 11:30 am, healthy subjects showed higher *wanting* for fat-rich foods than did HD patients on days with and without dialysis (**Figure 3B**).

Blood parameters (Table 3)

The mean plasma concentration of ghrelin was higher in HD patients at 8:00 am than at 11:00 am, and also higher than the concentration measured at 8:00 am in healthy subjects. The mean plasma leptin concentration decreased from 8:00 am to 11:00 am for all participants, particularly for HD patients. The mean plasma insulin concentration decreased from 8:00 am to 11:00 am for all participants.

From 8:00 am to 11:00 am, plasma AA concentrations decreased in HD patients and healthy subjects. Delta values indicated greater decreases in HD patients than in healthy subjects for glutamine, citrulline, cysteine, histidine, 1-3 methyl-histidine, lysine, arginine and proline. In contrast, smaller decreases were found in HD patients than in healthy subjects for plasma aspartic acid, serine and glutamine. Only tryptophan was lower in HD patients than in healthy subjects for both time points. Finally, at 8:00 am, mean citrulline, 1-3 methyl-histidine concentrations were higher in HD patients than in healthy subjects, while at 11:00 am, mean lysine and arginine concentrations were lower in HD patients.

Correlations between wanting for protein-rich foods and other parameters

In HD patients, the increase in *wanting* for protein-rich foods from 7:30 am to 11:30 am correlated with the decrease in plasma concentrations of glutamine (r=0.457, P< 0.05), alanine (r=0.559, P< 0.01), citrulline (r=0.422, P< 0.05), ornithine (r=0.591, P< 0.01) and proline (r=0.675, P< 0.001) and with the global decrease in plasma AAs (sum of all AAs) (r=0.574, P< 0.01). Conversely, the increase in *wanting* for protein-rich foods did not correlate with changes in plasma concentrations of ghrelin, leptin and insulin, hunger, overall *liking* and *wanting* for fat- and carbohydrate-rich foods.

In contrast, the increase in w*anting* for protein-rich foods in healthy subjects from 7:30 am to 11:30 am did not correlate with changes in plasma concentrations of AAs (taken individually or together) or plasma hormones, but it did correlate with an increase in hunger (r=0.757, P<0.001), *liking* for protein-rich foods (r=0.695, P<0.01) and *wanting* for fat- (r=0.553, P<0.05) and carbohydrate-rich foods (r=0.813, P<0.001).

When all variables, including hunger, *liking*, *wanting*, plasma ghrelin, leptin, insulin and AAs, were put into a principal component analysis there was a clear difference between HD patients and healthy subjects in *wanting* for protein-rich foods. In HD patients, *wanting* for protein-rich foods was mainly associated with the concentration of plasma AAs and not with hunger or *liking* or *wanting* for other types of foods (**Figure 4A**). The contrary was observed in healthy subjects (**Figure 4B**).

EXP2

Participants' characteristics (Table 4)

HD patients and healthy subjects were similar in terms of sex, age and BMI. Participants' mean body weight was stable during the six months preceding the study.

Plasma albumin was lower in HD patients than in healthy subjects, whereas transthyretin was similar in both groups. C-reactive protein was higher in HD patients than in healthy subjects (Table 4).

The mean normalized protein catabolic rate (nPCR) was 1.03±0.4 g/kg/day, and mean KT/V was 1.38±0.27 g/kg/day.

Hunger and liking before the meal

Hunger at about 12:30 pm was similar in all participants (**Figure 5**).

HD patients (days with and without dialysis) and healthy subjects showed similar *liking* for protein-, carbohydrate- and fat-rich dishes, but the low-energy dish (ratatouille) was preferred by healthy subjects (Table 5).

Food intake from the buffet (Table 6 and Figure 6)

HD patients ate more protein on the day with than on the day without dialysis, but there was no significant difference in energy, fat or carbohydrate intake between the two days.

The ratio of energy intake from protein was therefore higher just after dialysis than on the day without dialysis, while it was slightly lower for carbohydrates.

In healthy subjects, energy intake was lower than in HD patients. Healthy subjects consumed less protein than HD patients after dialysis and less fat than HD patients on both days. Carbohydrate intake was similar in all participants (healthy subjects and HD patients).

Postprandial hunger and liking for the eight dishes

After lunch, hunger was similar in all participants (**Figure 5**).

Compared with healthy subjects, HD patients reported a persistent higher *liking* for roast chicken after the meal on the dialysis day and, a lower *liking* for ratatouille on the day without dialysis (Table 5).

According to the questionnaires, the pleasantness procured by the foods, the variety of the dishes, the quantity proposed and the reasons for the subjects' choices were rated equally by all participants. The only difference was that healthy subjects were more likely to make food choices for hedonic reasons.

GENERAL DISCUSSION

This study showed that dialysis induced a significant increase in *wanting* for protein, which was higher on days with than on days without dialysis, bringing it up to levels seen in healthy subjects. This increase in the desire to eat protein-rich foods was confirmed by a significant increase in spontaneous protein intake just after dialysis.

In EXP1, according to the TFEQ, HD patients had a significantly more restrictive, less disinhibited and less hungry psychological profile than the healthy subjects, probably due to the dietary constraints usually required (33, 34). However, energy intake did not differ significantly between HD patients and healthy subjects. Furthermore, neither group showed significant PEW (similar BMI, weight and plasma albumin), although protein intake was slightly lower in HD patients and even though previous studies have shown that PEW is common in CKD patients (2, 3). As hunger at 11:30 am was similar in all participants, the observed changes in eating approaches, particularly in *wanting* for protein, cannot be related to differences in this parameter.

Before lunch, *wanting* for protein-rich foods in HD patients differed from that in healthy subjects. To our knowledge, decreased *wanting* for protein on days without dialysis (*i.e.,* at midday on the day between usual dialysis sessions) and the return to a normal level immediately after dialysis have never been reported. This lower *wanting* for protein 24 h before and increased *wanting* after dialysis indicates a change in the food reward system and reinforces data on changes in food preferences observed in HD patients: reduced preference for sweet food and refusal of red meat (28-31).

The increase in the desire to eat protein after dialysis (*i.e.* the increase in protein *wanting*) was confirmed by the results of EXP2. At lunch immediately after dialysis, the spontaneous *ad libitum* intake of protein in HD patients was higher than that in healthy subjects and higher than their own intake on the day without dialysis. It should be highlighted that differences in hunger cannot explain the increased protein intake after dialysis, as levels were similar before lunch for all participants.

There is an established link between impaired renal function and a gradual reduction in food consumption (27, 35). Decreased energy intake and protein-energy metabolic abnormalities appear to be the two main factors contributing to PEW in CKD patients (8). Though there are some benefits to increasing protein intake on days with dialysis (33-41), the present study supports the idea that protein-rich foods should be provided after dialysis since they are more appealing at that time (36-39). To our knowledge, no studies have observed an increase in the *wanting* and spontaneous intake of protein-rich foods after dialysis, but it should be noted that the use of protein-enriched meals during dialysis has been reported with beneficial effects. Indeed, in hypoalbuminemic hemodialysis patients, high-protein meals during dialysis combined with a potent phosphorus binder increases serum albumin while controlling phosphorus (40) and, patients report positive attitudes toward receiving high-protein-meals during dialysis without increasing symptomatic hypotension events (41). Further studies could compare the effect of protein-rich foods during and after dialysis on the food reward system and the nutritional status of HD patients.

The post-dialysis increases in *wanting* for protein does not seem to be a consequence of variations in plasma ghrelin, leptin and insulin concentrations since: 1) there was no correlation between increases in *wanting* for protein from 8:00 am to 11:00 am and changes in plasma hormone concentrations were observed; 2) principal component analyses showed very different statistical relationships between study outcomes for HD patients and healthy subjects for plasma ghrelin, leptin and insulin.

The post-dialysis increases in *wanting* for protein and protein consumption could be related to the change in plasma AAs. Fluctuations in plasma AAs may simultaneously affect the reward system, food motivation and food choices with an appetite for or a rejection of proteins (27, 42). These fluctuations can also influence the intake of protein-rich foods to restore or maintain an adequate protein status (43). In the present study, there was a significant decrease in almost all AAs during dialysis. As already shown by Boirie et al., loss of kidney function may also impair the conversion of some AAs, such as the conversion of phenylalanine to tyrosine (44). Therefore, the hemodialysis-mediated decrease in some AAs may remove the physiological “brake” they exert on *wanting* for protein when their concentrations are high. Principal component analyses support this interpretation (the increase in *wanting* for protein at lunch just after dialysis correlated only with decreases in plasma AA concentrations).

It is difficult to clarify the influence of each AA on the increase in *wanting* for protein after dialysis. Plasma tryptophan (Trp) could play a role. It was the only plasma AA whose concentrations were lower in HD patients than in healthy subjects at both 8:00 am and 11:00 am, as already reported (27, 45). Animal and human studies have shown that increases in plasma tryptophan concentrations or in the plasma tryptophan/large neutral AAs (*i.e.*, valine, isoleucine, leucine, phenylalanine, and tyrosine) ratios are related to increases in tryptophan concentrations in the central nervous system (42, 46, 47). This imbalance could interfere with the passage of hemato-meningeal branched-chain amino acids and Trp, with a subsequent increase in serotonin synthesis leading to anorexia (45). Further analysis of aminograms from dialysis liquids should help to determine the influence of each AA in the post-dialysis increase in *wanting* for protein.

For the purpose of this proof-of concept study, we deliberately selected a small number of homogenous patients without diabetes (diabetic patients often follow a restrictive diet that can modify their spontaneous feeding behavior, their taste perception and their food preferences). Consequently, these results cannot be generalized to all hemodialysis patients in particular to older and diabetic subjects, before supplemental experimental observations. Furthermore, as the present study was conducted over a short period it would be relevant to evaluate the long-term effect of protein supplementation just after dialysis to evaluate the acceptance of such diet and its effect on nutritional status in particular in HD patients with PEW assessed by the standard methods of SGA or Malnutrition Inflammation Score. Strength of the present study is the confirmation of the desire to eat protein-rich foods after dialysis as noted in EXP.1, under real-life conditions during a lunch buffet in uninformed subjects as observed in EXP. 2. In contrast, a limitation of the study consists in the absence of measurement of plasma AAs on the interdialytic interval day in Exp. 1 (initially, authors estimated that it was neither desirable nor ethical to take two blood samples on these days to avoid blood vessel break-ins).

In conclusion, the feeding behavior of HD patients changed immediately after hemodialysis, and probably during the dialysis given the anticipation of the amino acid depletion that happened with hemodialysis treatment, with significant increases in both *wanting* for and spontaneous *ad libitum* consumption of protein. These changes appear to be related to per-dialysis changes in plasma AA concentrations and not to variations in plasma ghrelin, leptin or insulin concentrations. This observation suggests that a post-dialysis meal of protein-rich food might be a key opportunity for HD patients to increase protein intake, nutritional status, quality of life and survival.

Practical Application:

- Protein-energy wasting is a risk factor for mortality and complications in hemodialysis patients
- The appetite/incentive motivation (*i.e.* the *wanting* component of the reward system) to eat protein-rich foods and the spontaneous *ad libitum* intake of protein-rich foods in real condition are high immediately after dialysis
- Increases in *wanting* and spontaneous intake of protein-rich foods correlated with the decrease in plasmatic amino acids concentrations by dialysis
- PEW could be prevented in HD patients by protein intake during and immediately after haemodialysis

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LEGENDS FOR FIGURES

Figure 1. Hunger sensation in 24 healthy subjects and in 24 hemodialysis patients on a dialysis day or an interdialytic interval day in Experiment 1.

Hunger was evaluated at 7:30 am and at 11:30 am, before and after the dialysis (50% of the patients were evaluated the day before and 50% the day after dialysis) or at an identical time when no dialysis was performed. Participants indicated their hunger sensation using a 10-cm visual analog scale. Values are means ± SDs. Hunger was higher at 11:30 am than at 7:30 am in healthy subjects ($P< 0.001$) and in hemodialysis patients ($P< 0.001$). At 7:30 am, hunger was similar in hemodialysis patients on both days but was higher in hemodialysis patients on the dialysis day than it was in healthy subjects ($P< 0.05$). At 11:30 am, hunger was similar in all subjects. * and *** indicate significant differences ($P< 0.05$ and $P< 0.001$, respectively).

Figure 2. The degree of *liking* of protein-, fat- and carbohydrate-rich foods in 24 healthy subjects and in 24 hemodialysis patients on a dialysis day or an interdialytic interval day in Experiment 1.

Liking (*i.e.,* the pleasantness induced by foods) was evaluated at 7:30 am and at 11:30 am for each food after smelling 2 protein-rich foods [Bündnerfleisch (dried beef) and imitation crab meat – Figure 2A], 2 fat-rich foods (melted butter and mayonnaise – Figure 2B) and 2 carbohydrate-rich foods (honey and strawberry jam – Figure 2C). *Liking* was evaluated using 10-cm VAS. Values are means ± SDs. The degree of *liking* of protein-rich foods increased from 7:30 am to 11:30 am in healthy subjects ($P< 0.001$) and in hemodialysis patients ($P<0.05$). The degree of *liking* of fat-rich foods increased from 7:30 am to 11:30 am only in healthy subjects ($P< 0.05$). The degree of *liking* for carbohydrate-rich foods did not change from 7:30 am to 11:30 am in healthy subjects and in hemodialysis patients. No significant difference in *liking* for each macronutrient was noted at 7:30 am and at 11:30 am in healthy subjects and hemodialysis patients.

Figure 3. The degree of *wanting* of protein, fat- and carbohydrate-rich foods in 24 healthy subjects and in 24 hemodialysis patients on a dialysis day or an interdialytic interval day in Experiment 1.

Wanting was evaluated at 7:30 am and at 11:30 am according to the desire to eat 6 protein-rich foods presented on separate pictures (fried eggs, grilled salmon, baked chicken, veal steak, rib steak and turkey breast – Figure 3A), 6 fat-rich foods presented on separate pictures (avocados, olives, peanuts, chocolate, fried fritters and whipped cream – Figure 3B) and 6 carbohydrate-rich foods presented on separated pictures (potato puree, rice, lentils, apples, pasta, and fruit cake – Figure 3C). *Wanting* was evaluated using 10-cm VAS. Values are means ± SDs. The degree of *wanting* of protein-, fat- and carbohydrate-rich foods increased from 7:30 am to 11:30 am in healthy subjects and in hemodialysis patients ($P < 0.001$ for all). A significant interaction and Tukey's *post hoc* tests indicated that the hemodialysis patients' *wanting* of protein-rich foods at 11:30 am was lower on the interdialytic day ($P < 0.01$) than it was on the dialysis day, and it was also lower ($P < 0.001$) than that of healthy subjects. No difference was observed in the degree of fat and carbohydrate *wanting* at 11:30 am on days with or without dialysis. Higher *wanting* of fat-rich foods was also noted in healthy subjects compared with that of hemodialysis patients both on the dialysis day and on the interdialytic interval day ($P < 0.05$, for both). *, ** and *** indicate $P < 0.05$, $P < 0.01$ and $P < 0.001$, respectively.

Figure 4. Principal component analysis with the variations of the measured parameters from the early to late morning (delta values) in 24 healthy subjects and 24 hemodialysis patients on a dialysis day in Experiment 1.

The principal component discriminates between the influences of the different parameters measured by the *wanting* of protein-rich foods (the desire to eat protein-rich foods after presentation of 8 pictures) in hemodialysis patients on the day of dialysis (Figure 4A) and in healthy subjects (Figure 4B). In hemodialysis patients, *wanting (W)* of protein-rich foods mainly correlated with a decrease in plasma amino acid concentrations but not with hunger sensation, *liking (L)* of protein, fat or carbohydrates, *wanting* of fat- and carbohydrate-rich foods or plasma leptin or insulin concentrations. In healthy subjects, *wanting* of protein-rich foods was positively related to hunger sensation, *liking* of all foods (proteins, fat and carbohydrates), and *wanting* of fat- and carbohydrate-rich foods; it was negatively correlated with plasma leptin and insulin concentrations but not with plasma amino acid concentrations.

Figure 5. Hunger sensation in 18 healthy subjects and in 18 hemodialysis patients on a dialysis day and on an interdialytic interval day before and after meal ingestion from a lunch buffet in Experiment 2.

Hunger was evaluated before and after a buffet composed of 8 courses (roast chicken, roast beef, smoked salmon rolled in St-Môret cheese, tuna and tomato salad, mayonnaise, cream of lentil soup, ratatouille and black cherries baked in a batter), using a 10-cm visual analog scale. Participants were able to eat each of the foods of their choice in the quantities they desired, and they were unaware of the objectives of the study. Values are means ± SD. Hunger decreased after ingestion of the meal in healthy subjects ($P < 0.001$) and in hemodialysis patients ($P < 0.001$).

Figure 6. Protein, fat and carbohydrate intake (g) from an 8-course lunch buffet served to 18 healthy subjects and 18 hemodialysis patients on a dialysis day and on an interdialytic interval day in Experiment 2.

Values are means ± SD. Protein intake was higher just after dialysis than it was on an interdialytic interval day ($P < 0.01$) but intakes of fat and carbohydrates were similar. Healthy subjects ate less protein than the hemodialysis patients did just after dialysis ($P < 0.05$), and they ate less fat ($P < 0.01$) but similar amounts of carbohydrates as hemodialysis patients in general.

Figure 1:

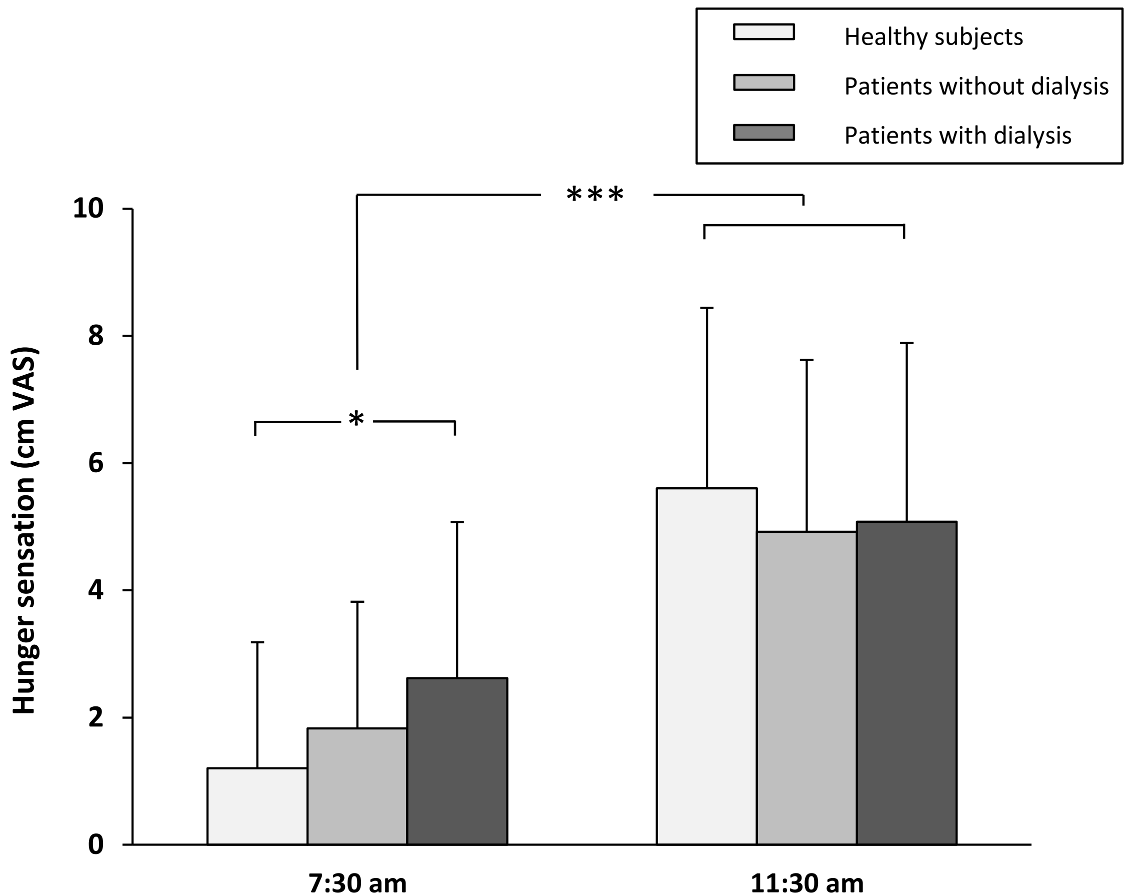
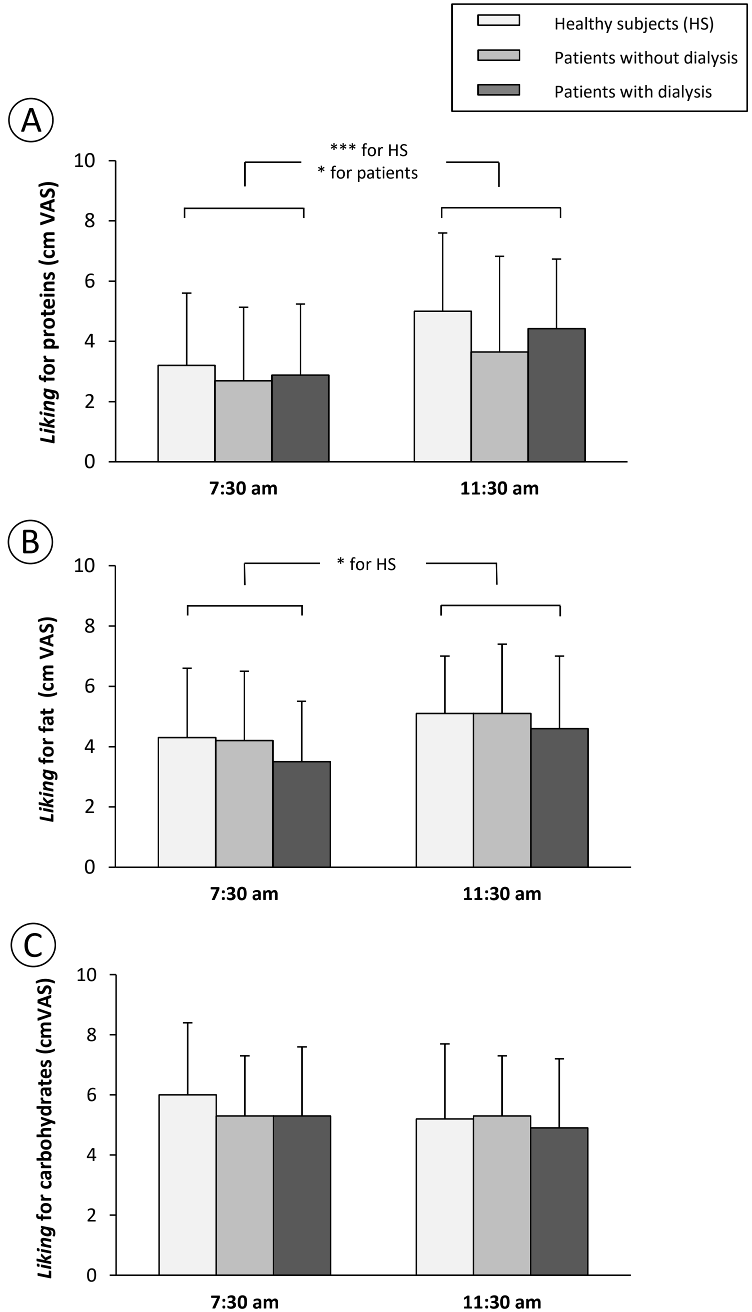

Healthy subjects
Patients without dialysis
Patients with dialysis
Hunger sensation (cm VAS)
10
8
6
4
2
0
***
*
7:30 am
11:30 am

Figure 2:



Healthy subjects (HS)
Patients without dialysis
Patients with dialysis
A
*** for HS
* for patients
Liking for proteins (cm VAS)
10
8
6
4
2
0
7:30 am
11:30 am
B
* for HS
Liking for fat (cm VAS)
10
8
6
4
2
0
7:30 am
11:30 am
C
Liking for carbohydrates (cmVAS)
10
8
6
4
2
0
7:30 am
11:30 am

Figure 3:

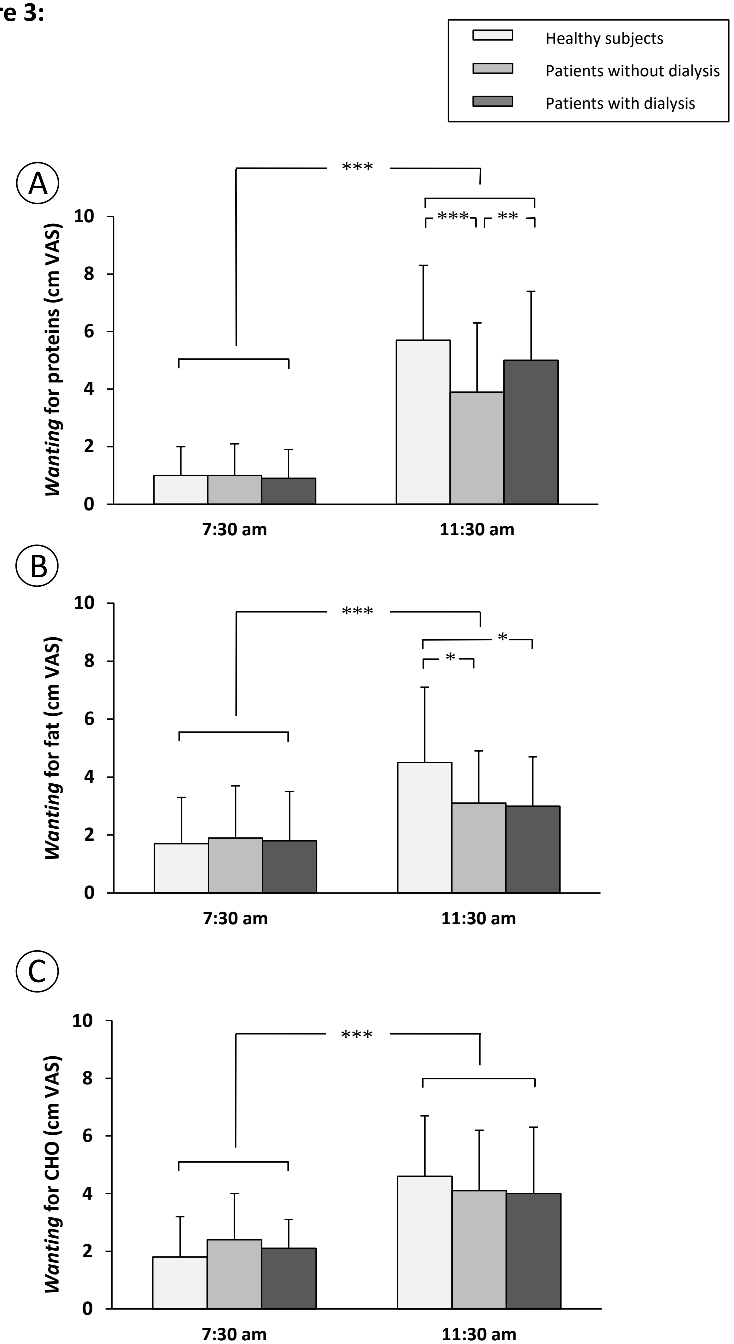
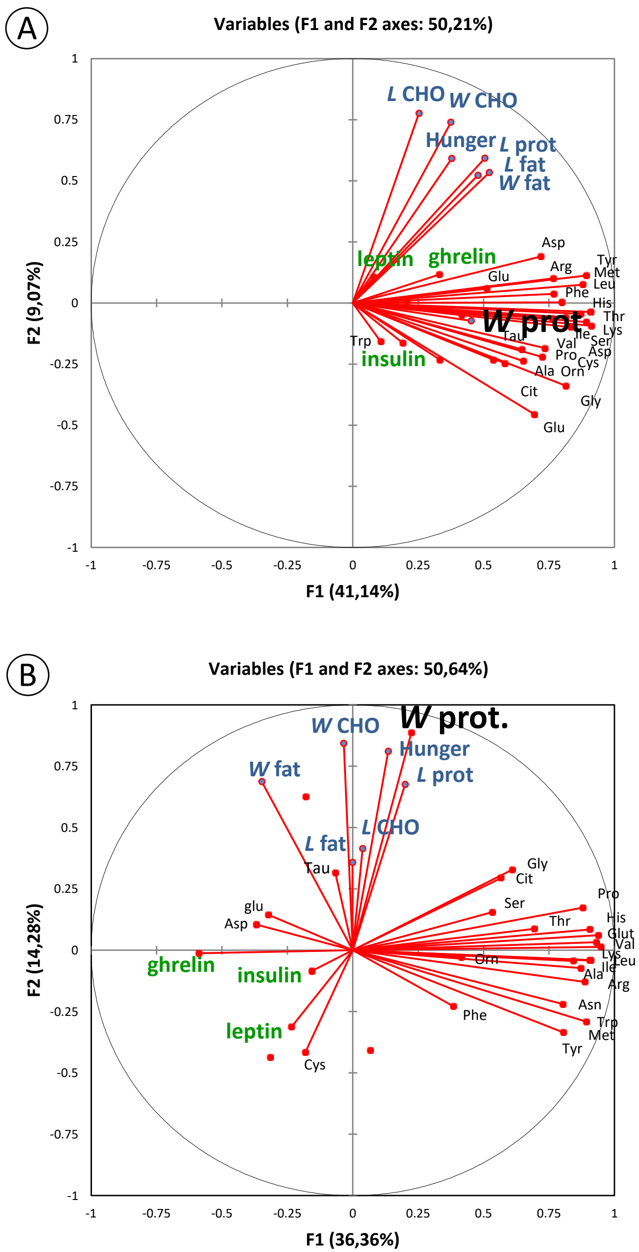
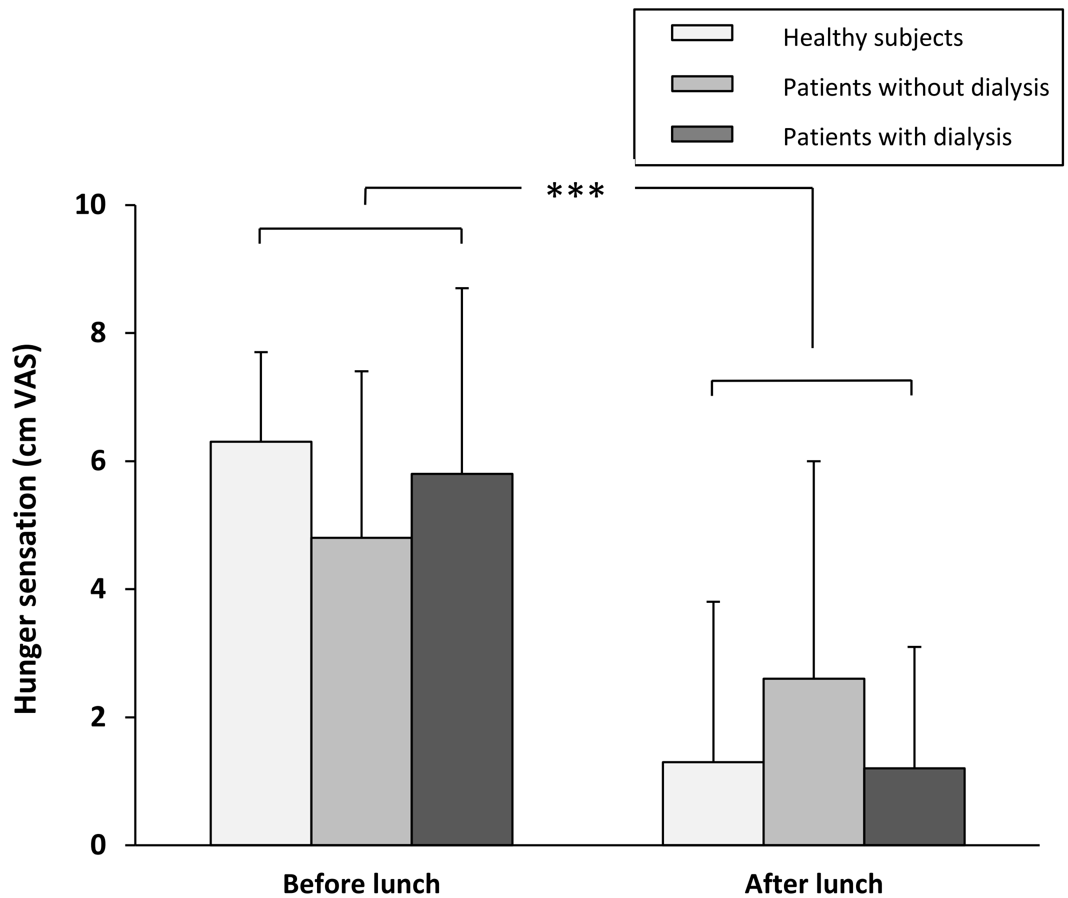

Healthy subjects
Patients without dialysis
Patients with dialysis
A
Wanting for proteins (cm VAS)
***
**
10
8
6
4
2
0
7:30 am
11:30 am
B
Wanting for fat (cm VAS)
*
C
Wanting for CHO (cm VAS)

Figure 4:



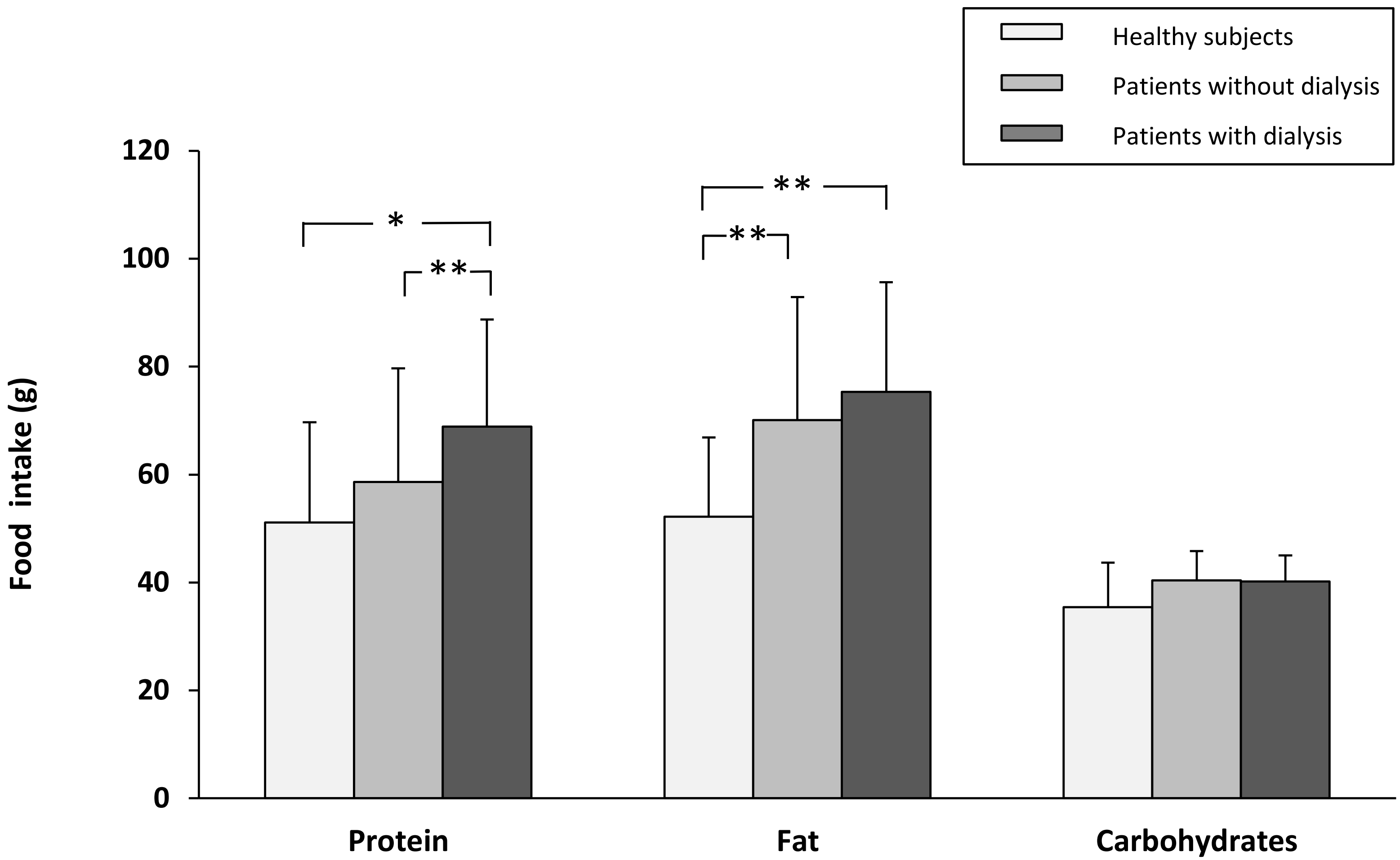
A
Variables (F1 and F2 axes: 50,21%)
F2 (9,07%)
F1 (41,14%)
L CHO
W CHO
Hunger
L prot
L fat
W fat
leptin
ghrelin
insulin
W prot
Asp
Arg
Glu
Tyr
Met
Leu
Phe
His
Thr
Lys
Ile
Ser
Asp
Tau
Val
Pro
Cys
Ala
Orn
Cit
Gly
Glu
Trp
B
Variables (F1 and F2 axes: 50,64%)
F2 (14,28%)
F1 (36,36%)
W prot.
W CHO
Hunger
L prot
W fat
L CHO
L fat
Tau
glu
Asp
ghrelin
insulin
leptin
Cys
Gly
Cit
Ser
Thr
Pro
His
Glut
Val
Lys
Leu
Orn
Ile
Ala
Arg
Asn
Phe
Trp
Met
Tyr

Figure 5:



Healthy subjects
Patients without dialysis
Patients with dialysis
***
10
8
6
4
2
0
Hunger sensation (cm VAS)
Before lunch
After lunch

Figure 6:



Healthy subjects
Patients without dialysis
Patients with dialysis
120
100
80
60
40
20
0
Food intake (g)
*
**
**
**
Protein
Fat
Carbohydrates

Table 1: Anthropometric characteristics and blood parameters in 24 healthy subjects and 24 hemodialysis patients before dialysis in Experiment 1.

	Healthy subjects	**Hemodialysis patients**
Sex (male/female)	7/17	7/17
Age (years)	59 ± 14	61 ± 13
Weight (kg)	76.2 ± 14.3	74.0 ± 16.5
Body mass index ($kg.m^{-2}$)	25.11 ± 3.42	25.85 ± 5.35
Hemoglobin (g/100 mL)	13.9 ± 1.3	11.7 ± 1.3***
Albumin (g/L)	38.6 ± 5.3	36.5 ± 7.0
Transthyretin (g/L)	0.270 ± 0.063	0.334 ± 0.0.97**
Protein (g/L)	72 ± 6	71 ± 8
Urea (mmol/L)	6.4 ± 1.9	24.4 ± 8.6***
Creatinine (µmol/L)	78 ± 15	847 ± 184***
Creatinine clearance (mL/min)	90.2 ± 15.7	5.2 ± 1.4***
Potassium (mmol/L)	4.1 ± 0.4	4.4 ± 0.6
Calcium (mmol/L)	2.21 ± 0.12	2.22 ± 0.18
Phosphorus (mmol/L)	1.02 ± 0.18	1.60 ± 0.50***
C-reactive protein (mg/L)	2.6 ± 5.6	16.0 ± 20.2***

Blood samples were taken at approximately 8:00 am. All values are means ± SDs. The Mann-Whitney U-test indicates significant differences between hemodialysis patients and healthy subjects (** and *** symbolize $P < 0.01$ and $P < 0.001$, respectively).

Table 2: Attitude towards food according to the Three-Factor Eating Questionnaire (TFEQ) and mean daily food intake from a 3-day dietary survey of 24 healthy subjects and 24 hemodialysis patients in Experiment 1.

	Healthy subjects	**Hemodialysis patients**
TFEQ – restriction	6.8 ± 3.7	9.1 ± 3.8*
TFEQ – disinhibition	6.0 ± 3.5	3.7 ± 1.7**
TFEQ – hunger	3.8 ± 3.0	1.8 ± 1.9*
Total energy intake (kJ/d) (kcal/d) (kcal/kg)	7163 ± 427 1712 ± 102 23.2 ± 4.5	6937 ± 2280 1658 ± 545 22.8 ± 7.2
Protein intake (kcal/d) (g/d) (% of energy intake) (g/kg bodyweight)	302 ± 25 76 ± 6 17.7 ± 0.7 1.03 ± 0.20	277 ± 76* 69 ± 19* 17.1 ± 2.5 0.96 ± 0.25
Carbohydrate intake (kcal/d) (g/d) (% of energy intake)	829 ± 49 207 ± 12 48.5 ± 1.8	757 ± 304* 189 ± 76* 44.9 ± 4.8**
Fat intake (kcal/d) (g/d) (% of energy intake)	578 ± 45 64 ± 5 33.8 ± 1.7	626 ± 202 70 ± 22 38.1 ± 4.2***

Before the experimental sessions, participants completed the TFEQ to evaluate their attitude toward food according to three dimensions (dietary restriction, disinhibition and hunger). After the experimental sessions, a dietary survey was conducted by a dietitian to assess participants' usual 24 h dietary intake. All values are means ± SDs. The Mann-Whitney U-test indicates significant differences between hemodialysis patients and healthy subjects (* and ** symbolize $P < 0.05$ and $P < 0.01$, respectively).

Table 3: Hormones and plasma amino acid profiles in 24 healthy subjects and 24 in hemodialysis patients in Experiment 1.

	Healthy subjects		**Hemodialysis patients**		
	8:00 am (A)	**11:00 am (B)**	**8:00 am (C)**	**11:00 am (D)**	**Statistics**
Ghrelin (pg/mL)	626 ± 361	890 ± 426	1659 ± 1191	974 ± 710	A-C* & C-D***
Leptin (ng/mL)	12.3 ± 12.9	11.8 ± 13.6	40.0 ± 45.4	25.6 ± 29.8	(1) and C-D***
Insulin (mUI/L)	28.7 ± 47.2	3.8 ± 5.5	30.1 ± 25.3	7.4 ± 7.5	(1)
Amino acids (nmol/ml)					(1)
Glycine	324.3 ± 91.8	256.8 ± 78.6	304.6 ± 147.2	217.4 ± 85.0	
Alanine	596.7 ± 205.5	418.1 ± 144.4	450.5 ± 260.8	235.8 ± 131.2	
Serine	166.4 ± 44.1	121.2 ± 31.4	105.0 ± 31.7	80.0 ± 19.4	A-B* & C-D***
Threonine	164.6 ± 63.8	131.6 ± 41.1	121.7 ± 50.6	75.0 ± 28.8	
Valine	356.8 ± 104.8	292.1 ± 78.0	235.5 ± 49.4	153.7 ± 26.6	
Isoleucine	96.4 ± 40.6	69.5 ± 20.4	73.5 ± 26.4	52.3 ± 19.5	
Leucine	194.1± 74.5	145.5 ± 37.1	123.6 ± 33.8	96.4 ± 25.6	
Lysine	247.4 ± 79.5	215.1 ± 71.4	183.5 ± 58.3	110.2 ± 39.8	B-D* & C-D***
Arginine	114.1 ± 32.2	119.7 ± 22.9	109.8 ± 31.3	76.1 ± 26.2	B-D* & C-D***
Histidine	107.8 ± 29.0	90.4 ± 22.5	81.3 ± 25.3	49.1 ± 12.6	C-D***
Tyrosine	97.7 ± 33.2	76.4 ± 27.7	55.8 ± 17.3	35.0 ± 11.6	
Phenylalanine	90.7 ± 27.9	71.1 ± 16.1	79.0 ± 21.6	58.1 ± 15.5	
Tryptophan	78.0 ± 23.5	50.3 ± 14.7	34.7 ± 13.2	16.2 ± 6.8	(2)
Methionine	40.3 ± 12.5	27.7 ± 8.0	27.8 ± 8.0	19.3 ± 6.3	
Cysteine	43.2 ± 22.6	22.8 ± 6.8	78.0 ± 29.1	17.8 ± 6.7	C-D***
Proline	331.6 ± 102.0	247.2 ± 60.3	431.5 ± 174.7	231.4 ± 83.8	C-D***
Glutamine	669.7 ± 184.4	609.8 ± 172.4	622.5 ± 267.5	404.6 ± 141.6	C-D***
Glutamic acid	92.2 ± 46.5	48.4 ± 28.1	118.8 ± 75.3	105.0 ± 39.9	
Asparagine	92.0 ± 27.4	72.9 ± 20.1	74.0 ± 22.0	48.2 ± 16.2	
Aspartic acid	26.4 ± 11.7	16.2 ± 9.9	24.0 ± 9.8	21.9 ± 8.0	
Citrulline	42.2 ± 17.0	40.1 ± 9.9	99.8 ± 38.3	41.7 ± 12.7	A-C*** & C-D***
Ornithine	113.5 ± 29.9	71.6 ± 27.6	109.9 ± 39.7	65.4 ± 31.2	
Taurine	192.2 ± 73.6	92.5 ± 29.1	169.2 ± 60.5	96.6 ± 58.3	

Blood samples were drawn at approximately 8:00 am in hemodialysis patients (when connected to the hemodialysis apparatus) and were drawn again at approximately 11:00 (when disconnected from the hemodialysis apparatus). Blood was also collected at the same time from healthy subjects. All values are means ± SDs. Capital letters refer to the following: A, healthy subjects - 8:00 am; B, heathy subjects - 11:00 am; C, hemodialysis patients - 8:00 am; and D, hemodialysis patients - 11:00 am. Two-way repeated measure ANOVAs indicate, 1) significant decreases from 8:00 am to 11:00 for plasmatic concentrations of leptin ($P<0.01$), insulin ($P< 0.001$) and all amino acid ($P< 0.001$, except $P< 0.01$ for arginine), as showed by (1); 2) lower plasmatic concentration of tryptophan in hemodialysis patients compared with healthy subjects ($P< 0.05$) as pointed by (2) and, 3) significant interactions highlighted with Tukeys' post hoc tests as represented by stars (* and *** symbolize $P< 0.05$ and $P< 0.001$, respectively). For additional information on the statistical results, see supplemental material 3.

Table 4: Anthropometric characteristics and blood parameters in 18 healthy subjects and in 18 hemodialysis patients before dialysis in Experiment 2.

	Healthy subjects	**Hemodialysis patients**
Sex (male/female)	9/9	10/8
Age (years)	68 ± 12	69 ± 11
Weight (kg)	76.2 ± 14.3	74.0 ± 16.5
Body mass index ($kg.m^{-2}$)	25.76 ± 4.35	26.04 ± 5.81
Albumin (g/L)	42.1 ± 2.4	37.7 ± 3.9**
Transthyretin (g/L)	0.286 ± 0.038	0.323 ± 0.083
Urea (mmol/L)	6.2 ± 0.9	A
Creatinine (µmol/L)	80 ± 13	A
Creatinine clearance (mL/min)	77.3 ± 11.3	A
C-reactive protein (mg/L)	1.9 ± 1.5	23.0 ±52.4***

Healthy subjects were matched for sex, age and body mass index to hemodialysis patients. Blood samples were drawn at approximately 9:00 am. All values are means ± SDs. The Mann-Whitney U-test indicates significant differences between hemodialysis patients and healthy subjects (** and *** symbolize $P< 0.01$ and $P< 0.001$, respectively). “A” indicates that these values were not pertinent in hemodialysis patients.

Table 5: *Liking* before and after intake of the 8 courses in 18 healthy subjects and in 18 hemodialysis patients just after dialysis and on an interdialytic day in Experiment 2.

	Healthy subjects	Hemodialysis patients without dialysis	hemodialysis patients after dialysis
Before lunch			
Roast chicken	6.1 ± 2.2	6.1 ± 2.4	7.0 ± 2.5
Roast beef	6.3 ± 1.4	6.0 ± 2.8	5.9 ± 3.0
Salmon rolled in cheese	5.6 ± 2.6	7.3 ± 2.6	6.7 ± 3.0
Tuna and tomato salad	5.2 ± 2.1	7.0 ± 2.3	6.5 ± 3.1
Mayonnaise	5.7 ± 1.8	5.9 ± 2.9	5.6 ± 3.1
Cream of lentil soup	7.2 ± 2.4	4.5 ± 3.3	5.4 ± 3.6
Black cherries baked in batter	6.8 ± 2.2	7.4 ± 2.5	7.3 ± 2.6
Ratatouille	6.4 ± 2.1[a]	3.3 ± 3.0[a]	4.6 ± 2.9
After lunch			
Roast chicken	3.9 ±2.9[a]	6.0 ± 2.5	6.6 ± 2.8[a]
Roast beef	6.0 ± 3.6	4.9 ± 3.3	5.0 ±3.2
Salmon rolled in cheese	5.1 ± 3.5	6.1 ± 2.1	5.8 ± 3.2
Tuna and tomato salad	6.6 ± 2.8	6.1 ± 2.9	6.6 ± 2.8
Mayonnaise	5.1 ± 3.7	4.9 ± 3.1	5.2 ± 3.4
Cream of lentil soup	5.5 ± 2.8	4.7 ± 3.2	4.6 ± 3.7
Black cherries baked in batter	7.6 ± 2.9	7.7 ± 2.3	7.5 ± 2.9
Ratatouille	5.8 ± 2.6[b]	2.6 ± 2.6[b]	3.9 ± 3.3

All values are means ± SDs. Results with the same letter next to them indicate that they are significantly different. Then, before and after intake, ratatouille was less liked by hemodialysis patients compared to healthy subjects on the interdialytic day ; after intake and dialysis, a higher degree of *liking* for roast chicken persisted in hemodialysis patients compared with healthy subjects (a and b indicates respectively, $P < 0.05$ and $P < 0.01$). For additional information on the statistical results, see supplemental material 3.

Table 6: Food intake at an 8-course lunch buffet for 18 healthy subjects and 18 hemodialysis patients just after dialysis and on an interdialytic day in Experiment 2.

	Healthy subjects	**Hemodialysis patients, interdialytic**	**hemodialysis patients, after dialysis**
Energy intake (kJ)	3422 ± 900[a,f]	4297 ± 1243[a]	4661 ± 1063[f]
(kcal)	816 ± 215[a,f]	1027 ± 297[a]	1114 ± 254[f]
Protein intake (g)	51 ± 19[d]	59 ± 21	69 ± 20[d],**
(% of energy intake)	24.6 ± 4.4	22.5 ± 3.5	24.6 ± 3.7***
(g/kg bodyweight)	0.72 ± 0.32[d]	0.84 ± 0.31	0.99 ± 0.29[d],**
Fat intake (g)	52 ± 15[b,f]	70 ± 23[b]	75 ± 20[f]
(% of energy intake)	25.6 ± 1.7[b,d]	27.1 ± 1.5[b]	26.8 ± 1.8[d]
Carbohydrate intake (g)	35 ± 8	40 ± 5	40 ± 5
(% of energy intake)	17.9 ± 4.6[d]	16.6 ± 3.5	15.0 ± 3.3[d],*
Roast chicken	74 ± 55[d]	80 ± 60	116 ± 46[d],**
Roast beef	72 ± 61	71 ± 57	90 ± 56
Salmon rolled in cheese	129 ± 54[a,e]	199 ± 84[a]	204 ± 65[e]
Tuna and tomato salad	91 ± 40	100 ± 45	101 ± 42
Mayonnaise	94 ± 72[b,e]	177 ± 101[b]	196 ± 115[e]
Cream of lentil soup	119 ± 57	135 ± 124	147 ± 102
Black cherries baked in batter	221 ± 63[c,f]	257 ± 16[c]	251 ± 33[f]
Ratatouille	16 ± 4[a,d]	9 ± 7[a]	10 ± 7[d]

All values are means ± SDs. Patients ate more protein from the buffet just after dialysis than they did on the interdialytic day, with no significant difference in energy content, fat and carbohydrate intake. The ratio of energy intake from protein to total energy intake was therefore higher after dialysis than it was on the interdialytic day, though it was similar for fat and lower for carbohydrates on the interdialytic day. In details (see supplemental material 3), compared with the interdialytic day, hemodialysis patients after dialysis ingested significantly more roast chicken and non-significantly (P=0.097), more roast beef, but similar amounts of the other courses. *, ** and *** indicate significant differences between the two days in hemodialysis patients with $P< 0.05$, $P< 0.01$ and $P< 0.001$, respectively. Healthy subjects differed from hemodialysis patients on the interdialytic day (results with the same letter indicate significant differences between those results, with a, b and c indicating $P< 0.05$, $P< 0.01$ and $P< 0.001$, respectively) and the day after dialysis (d, e and f indicate $P< 0.05$, $P< 0.01$ and $P< 0.001$, respectively).