Whole body protein metabolism in chronic hemodialysis
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Document Version
Publisher's PDF, also known as Version of record

Publication date:
2004

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Citation for published version (APA):

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Chapter 4

Protein intake during hemodialysis maintains a positive whole body protein balance in chronic hemodialysis patients

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Dirk-Jan Reijnoud
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Abstract

Background. Protein-energy malnutrition is present in 18 to 56% of hemodialysis patients. Since hemodialysis has been regarded as a catabolic event, we studied whether consumption of a protein and energy enriched meal improves the whole body protein balance during dialysis in chronic hemodialysis (CHD) patients.

Methods. Patients were studied on a single day between dialysis (HD- protocol) in the morning while fasting and in the afternoon while consuming 6 small test meals. Patients were also studied during two separate dialysis sessions (HD+ protocol). Patients were fasted during one and consumed the meals during the other. Whole body protein metabolism was studied by primed-constant infusion of L[1-13C]-valine.

Results. During HD feeding changed the negative whole body protein balance observed during fasting into a positive protein balance. Dialysis deepened the negative balance during fasting, whereas feeding during dialysis induced a positive balance comparable to the HD- protocol while feeding. Plasma valine concentrations during the studies were correlated to whole body protein synthesis and inversely correlated to whole body protein breakdown.

Conclusion. We conclude that the consumption of a protein and energy enriched meal by CHD patients while dialysing can strongly improve whole body protein balance, probably due to the increased amino acid concentrations in blood.

Keywords: Protein turnover, hemodialysis patients, stable isotope, valine, protein intake.
Introduction

Signs of protein-energy malnutrition occur frequently in patients with chronic renal failure (1;2). Protein-energy malnutrition has been shown to be a major risk factor for increased morbidity and mortality in the CHD patient (2;3). Multiple factors predispose CHD patients to protein-energy malnutrition e.g. low caloric intake, low protein intake and the hemodialysis procedure itself. Particularly losses of amino acids or abnormal protein metabolism during hemodialysis might contribute to the observed protein-energy malnutrition. Studies examining the role of CHD itself on protein metabolism are limited (4). Several lines of evidence indicate that the CHD procedure can result in a negative whole body protein balance. Nitrogen balance has been shown to be more negative on a dialysis day compared to a non-dialysis day regardless of daily protein intake (5;6). Lim et al. (4) studied whole body protein metabolism by applying the $^{13}$C-leucine isotope dilution technique in fasting CHD patients during hemodialysis. They observed a reduction in whole body protein synthesis compared to the pre-dialysis period and this resulted in a doubling of the negative protein balance already present in fasting CHD patients. Furthermore, hemodialysis stimulates muscle protein losses when compared to the pre-dialysis period in fasting CHD patients (7).

In apparently healthy subjects, the consumption of a meal or the administration of an amino acid mixture reverses the negative protein balance observed after an overnight fast (for reviews see(8;9)). Particularly, amino acids in plasma are powerful modulators of protein metabolism, as a mixture or in conjunction with insulin (10). Protein breakdown is inhibited while protein synthesis and protein oxidation are stimulated by amino acid infusion. As a result, whole body protein balance becomes positive (11;12).

The situation in CHD patients is less well known, and the effects of a meal during dialysis have not been studied so far. It is common clinical practice, at least in Europe, that CHD patients are allowed to eat during a 4 h. dialysis session. We adapted this practice for the purpose of nutritional intervention. A milk-based protein and energy enriched meal was given to the patients during a dialysis session and on a non-dialysis day. The meal was designed with the assumption that a maximum anabolic response would be elicited in our patients by a meal enriched in both energy and protein. We studied the effect of this oral intradialytic nutrition on whole body protein metabolism during hemodialysis with a biocompatible membrane in CHD patients. We addressed two questions more specifically: (i) to what extent does consumption of a protein and energy enriched meal result in a positive whole body protein balance in CHD patients and (ii) how effective is such a meal
consumed during a dialysis session in the prevention of the negative protein balance in CHD patients during dialysis. The first question was studied in CHD patients during a non-dialysis day, the second question during two dialysis sessions separated by 1 week. Whole body protein metabolism was studied applying stable isotope infusion techniques using [1-\textsuperscript{13}C]-valine as a tracer (13). The use of this tracer has certain advantages over [1-\textsuperscript{13}C]-leucine both analytically and metabolically. [1-\textsuperscript{13}C]-valine has been used previously by our laboratory in the study of whole body protein metabolism and synthesis of several specific proteins (14) in nephrotic patients. Furthermore, it has been reported that, under certain conditions, high (flooding) doses of leucine can provoke an insulinomimetic effect on protein metabolism (15;16) whereas this is not the case for valine. At doses normally applied in the study of whole body protein metabolism, valine and leucine give similar values of the fluxes of protein breakdown, synthesis and oxidation (17).

Subjects and methods

Study subjects

All non-diabetic stable hemodialysis patients, aged less than 65 years, in the Dialysis Centre Groningen were approached to participate in the two protocols of the present study i.e. a non-dialysis and a dialysis protocol. Twelve patients gave their permission but only three of them agreed to participate in both protocols. The other patients considered this too great a demand since they objected to fasting during the dialysis session. In summary, three patients participated in both protocols, six patients participated only in the non-dialysis protocol and three patients participated only in the dialysis protocol (table 1 patient number). The medical ethical committee of the University of Groningen approved all studies and written informed consent was obtained from all participants. All participants were clinically stable, without intercurrent acute illness in the three months before the study protocol and had been in dialysis for 6 months or more. The diagnoses were chronic glomerulonephritis in 3 patients (one with hypertension), nephropathy due to hypertension in 3 patients, quiescent Wegeners disease in 1 patient, polycystic kidney disease in 3 patients, and the cause of renal failure was unknown in 2 cases. Medications included phosphate binders, iron, multivitamins, antihypertensive drugs, calcitriol, and recombinant human erythropoietin of which the dose had not been altered for 3 months before the study protocol to avoid altered hematopoiesis. No patients received steroid hormones or immunosuppressive agents in the 6 months before the study protocol. The patients were dialysed with low flux biocompatible dialyzers for 4 hours three times weekly. Blood flow ranged from 250 to 350 ml min\textsuperscript{-1} and dialysate flow was 500 ml min\textsuperscript{-1}. 
Standard dialysate with 140 mEq Na$^+$, and 34 mEq bicarbonate was used for all patients. Glucose content in dialysate was 5.6 mM in 2 patients and 11.2 mM in 4 patients during their experimental dialysis sessions. Residual renal function was 3 ml min$^{-1}$ or less which corresponded to a Kt/V value of 0.45 week$^{-1}$ or less.

**Materials**

L[1-$^{13}$C] valine and NaH$^{13}$CO$_3$, both with an enrichment of >99% atom percent, were purchased from Cambridge Isotope Laboratories, Inc (Andover, MA, USA). Chemical purities were confirmed before use. Pyrogen and bacteria free solutions were prepared in sterile saline by the hospital dispensary the afternoon before the study day. Meal portions consisted of 150 grams of yoghurt (5.7 g protein, 7.4 g carbohydrate, and 5.4 g fat, Domo, The Netherlands), 20 grams of cream (0.5 g protein, 0.7 g carbohydrate, and 6.3 g fat Friesche vlag, Ede, the Netherlands) and 5 grams of protein enriched milk powder (1.5 g protein, 2.4 g carbohydrate, and 0.8 g fat, Fortify, Nutricia, The Netherlands). Consumption of a meal portion every 30 minutes for 3 hours resulted in a dietary valine intake of $132 \pm 20 \ \mu$mol kg$^{-1}$ h$^{-1}$ (18) and a fluid intake of 350 ml h$^{-1}$. The energy content of a meal portion was 386 kcal h$^{-1}$. Meals were designed to give > 50% of daily protein intake, $0.62 \pm 0.09$ gram kg$^{-1}$ of protein and $15 \pm 2$ kcal kg$^{-1}$ in energy content. It was assumed that gastric emptying during the meal was not disturbed since our patients had no history of dyspeptic symptoms during the three months before both protocols and were in a good nutritional state (19).

**Experimental design**

**Pilot experiments.** Dialysis by itself was found to increase the $^{13}$CO$_2$ enrichment in expired air gradually because of entrance of bicarbonate with a high natural enrichment from the dialysate (-4.0 $\pm$ 0.3 ‰ versus PDB limestone). Therefore, background enrichment in expired breath was measured independently in 5 patients during a dialysis session prior to this study. The time-course of this change as a percentage of the initial background enrichment of expired CO$_2$ was used to correct the value of the $^{13}$CO$_2$ excess enrichment obtained during either the $^{13}$C-bicarbonate or $^{13}$C-valine infusion for calculations of whole body protein metabolism. In a second pilot experiment it was tested to what extent the rate of $^{13}$C-valine infusion had to be increased during dialysis. This was deemed necessary since it was observed that the turnover in the bicarbonate pool was increased during dialysis and consequently, infusion of valine had to be increased to obtain $^{13}$CO$_2$ enrichments in expired air, which could be measured reliably in excess of background enrichment that had already been changed by exchange of plasma and dialysate bicarbonate. Doubling the [1-$^{13}$C]-valine infusion rate appeared to be sufficient.
Study protocols. The present study comprised two protocols. In the non-dialysis protocol (HD-), patients were studied on a day between two dialysis days. Fasting whole body protein metabolism was measured in the morning while fasting. This was followed in the afternoon by the measurement of whole body protein metabolism in patients consuming a protein and energy enriched meal in 6 portions (HD-fed). The dialysis protocol (HD+) could not be done on a single day and therefore consisted of two study days one week apart. Patients were dialysed normally on these days and measurements were made during the dialysis session. On one occasion, patients were studied while they remained fasting (HD+fas) and on the other occasion patients consumed a protein-enriched meal (HD+fed). The HD+ protocol started after the completion of the study of whole body protein metabolism during the HD- protocol. Three weeks prior to the study, all patients visited the Dialysis Centre Groningen for a dietary interview and instructions on dietary recording. Patients consumed a protein intake of $1.0 \pm 0.1 \text{ g kg}^{-1} \text{ day}^{-1}$ while caloric intake was not restricted.

Non-dialysis protocol (HD-). In the HD- protocol, patients had fasted overnight and were studied during a midweek day without dialysis, having dialysed the afternoon
before. Patients were admitted to the Hospital Research Unit at ≈ 7:30 AM. A catheter was inserted into the dorsal vein of the hand of the shunt arm to collect baseline blood samples. Subsequently breath samples were taken. A schematic diagram of the study day is shown in figure 1A. The NaH\textsubscript{13}CO\textsubscript{3} infusion was started at 8:00 AM. During the first hour whole body bicarbonate production (details explained in valine oxidation section) was measured using a primed constant infusion of NaH\textsubscript{13}CO\textsubscript{3} (5 μmol kg\textsuperscript{-1} bolus followed by a continuous infusion of 5 μmol kg\textsuperscript{-1} h\textsuperscript{-1}). Four breath samples were taken from 30 to 60 minutes after the start of the NaH\textsubscript{13}CO\textsubscript{3} infusion at 10 min intervals. The NaH\textsubscript{13}CO\textsubscript{3} infusion was discontinued immediately after the last breath sample was taken and the L[1-\textsuperscript{13}C]-valine infusion was started with a bolus of 15 μmol kg\textsuperscript{-1} followed by a continuous infusion of 7.5 μmol kg\textsuperscript{-1} h\textsuperscript{-1} for the next 4 hours. A second catheter was now inserted in the contralateral arm to collect blood samples. Blood and breath samples were taken simultaneously every half hour for 3 hours after the start of the \textsuperscript{13}C-valine infusion. During the fourth hour, blood and breath samples were taken every 15 minutes. At 1:00 PM, the meal period was started by consumption of the first portion of the protein-enriched meal and continued for 3 hours by consumption of a portion every 30 minutes. \textsuperscript{13}C-valine infusion continued at the same rate during this study period. Blood and breath samples were taken every 30 minutes for two hours after the start of the meal while during the last hour, samples were taken every 15 minutes. The study day ended at 4:00 PM, all catheters were removed and patients were observed until stable and then discharged.

**Dialysis protocol (HD\textsuperscript{+}).** In this protocol, patients were studied on two separate dialysis sessions, separated by 1 week. On one occasion patients were studied while they remained fasting. On the second occasion, patients consumed 6 small meals, the first 1 hour after the start of dialysis followed by 5 meals spaced by 30 minutes. Patients had been dialysed 44 ± 3 hours before they entered the study protocol. Studies were performed in the afternoon and patients consumed a late evening snack the evening before the study day to keep the fasting period comparable to that in the HD- protocol. Patients were admitted to the dialysis centre at ≈ 11:30 AM. Dialysis needles were inserted into the AV shunt to collect baseline blood samples and breath samples were simultaneously collected. Dialysis started at ≈ 12:00 PM. A primed continuous infusion of NaH\textsubscript{13}CO\textsubscript{3} was administered for one hour through the venous line of the dialysis machine (5 μmol kg\textsuperscript{-1} bolus followed by a continuous infusion of 5 μmol kg\textsuperscript{-1} h\textsuperscript{-1}) and four breath samples were taken from 30 to 60 minutes after the start of the infusion at 10 min intervals. The NaH\textsubscript{13}CO\textsubscript{3} infusion was discontinued after the last breath sample was taken and a primed continuous infusion of L[1-\textsuperscript{13}C] valine was started through the venous line of the dialysis machine for 3 hours (15 μmol kg\textsuperscript{-1} bolus followed by a continuous infusion of 15 μmol kg\textsuperscript{-1} h\textsuperscript{-1}). Blood samples
from the arterial line of the dialysis machine and breath samples were taken every half hour for the first two hours after the start of the $^{13}$C-valine infusion. During the third and last hour, blood and breath were sampled every 15 minutes (Fig. 1B). When whole body protein metabolism was studied during the meal period, the same experimental set-up was used as described above with the exception that, at the start of the $^{13}$C-valine infusion, the first of the 6 meal portions was consumed while the remaining 5 were consumed every 30 minutes during the next 3 hours (Fig. 1C). Blood pressure was monitored during all experimental dialysis sessions. Blood flow was estimated from the flow given on the dialysis machine while dialysate flow was 500 ml min$^{-1}$ plus the ultrafiltration. Approximately 70% of the ingested fluid was removed during the experimental dialysis session while the other 30% was removed during the next dialysis session.

**Analytical procedures**

Four ml of blood was drawn for each sample in liquid heparinized vacuum tubes and centrifuged at 3000 RPM. Plasma was extracted and stored at –20 ºC until analysis. Breath samples were collected in gas collection tubes using a straw as described earlier (20). Subjects exhaled normally through a straw into the glass container. After exhalation was completed, tubes were closed immediately and stored at room temperature until analysis. Dialysate was sampled every half hour using a syringe to extract 4 ml of dialysate and stored at –20 ºC until analysis.

Amino acid concentrations in plasma and spent dialysate were measured by AccQ Tag method using high-performance liquid chromatography (HPLC) according to the manufacturers protocols (Waters BV. Breda, The Netherlands). Amino acids (AA) were grouped according to total amino acids (TAA), the sum of the concentration of all individual amino acids, essential AA (the sum of the concentration of arginine, histidine, lysine, methionine, phenylalanine, threonine, iso-leucine, leucine, and valine), and the non-essential AA (the concentration of total AA -- essential AA). Insulin in plasma was determined by a double AB system as described earlier (21). Glucose and albumin concentrations were determined by standard clinical chemistry methods.

Measurement of $^{13}$CO$_2$ isotopic enrichment was performed by sampling directly the glass container with a Finnigan TracerMat (Finnigan MAT, San Jose, CA, USA) continuous flow isotope ratio mass spectrometer as described by Vonk et al. (20).

The determination of L-[1$^{13}$C] valine isotopic enrichment was done as described earlier (22). In short, amino acids were isolated from deproteinized plasma using a cation
exchange column (SCX-100, 209800, Alltech, Deerfield, IL, USA). The isolated amino acids were derivatized to their corresponding N(O)-methoxycarbonyl methyl ester (MCM) according to Husek (23). Analysis of isotopic enrichment of plasma $^{13}$C-valine was carried out by GC/MS on a Hewlett Packard 5890 Plus gas chromatograph coupled to a Finnigan SSQ 7000 quadruple mass spectrometer using methane positive-ion chemical ionisation. The gas chromatograph was fitted with a capillary column (AT 1701, length 20 m, id. 0.18 mm, film thickness 0.40 μm, Alltech, Deerfield, IL, USA). The mass spectrometer was operated in the selected ion monitoring mode at fragments m/z 190/191 of the $[\text{MH}]^+$ and $[\text{MH}+1]^+$ ions of the MCM derivative of unlabelled and L-[1-$^{13}$C]-valine respectively.

$[1-^{13}$C$]$ KIVA (alpha-keto-isovaleric acid) isotopic enrichment was determined according to Kulik et al. (24). In short, standards with a tracer mole ratio for $[1-^{13}$C$]$ KIVA ranging from 0 – 22 % were prepared by enzymatic conversion of standards L-[1-$^{13}$C$]$ valine with the corresponding tracer mole ratio as described earlier (25). Standards of $[1-^{13}$C$]$ KIVA and patient plasma samples were processed in the same series. KIVA was converted into its quinoxalinol-O-t-butyldimethylsilyl derivative. Isotopic enrichment of the derivatized samples was measured by GC/MS on a Hewlett Packard 5890 plus gas chromatograph coupled to a Finnigan SSQ 7000 quadruple mass spectrometer using positive ion electron impact ionisation. The gas chromatograph was fitted with a capillary column (Alltech, AT 1701). The mass spectrometer was operated in the selected ion monitoring mode recording fragments at $m/z$ 245 and 246 of unlabeled and $^{13}$C labelled KIVA respectively. All isotopic enrichments were measured against standard calibration curves.

*Evaluation of primary data*

Rate of appearance of intracellular valine $R(a)$ was calculated at isotopic steady state using the inverted pool model as described by Matthews et al. (26-28) for leucine kinetics. When this isotopic model is applied to $[1-^{13}$C$]$-valine, enrichment of plasma KIVA is assumed to provide an estimate of intracellular enrichment of valine. $R(a)$ in μmol valine kg$^{-1}$ h$^{-1}$ was calculated according to:

$$R(a) = \left( \frac{\text{MPEi(V)}}{\text{MPE(KIVA)}} - 1 \right) \times \text{i(V)}$$  

*eq(1)*

Where MPEi(V) is the isotopic enrichment of the valine in the infusate in mole percent excess, MPE(KIVA) is the isotopic enrichment of KIVA in plasma in mole percent excess, and i(V) is the infusion rate of [1-$^{13}$C]valine in μmol kg$^{-1}$ h$^{-1}$. 
Rate of oxidation of valine was calculated following the approach described by Van Goudoever \textit{et al.} (29). We did not use indirect calorimetry in our study to determine CO$_2$ production as a measure of whole body bicarbonate production. Measurements would be perturbed when the comparison between the HD- and the HD+ protocol is made because bicarbonate from the dialysis fluid enters the circulation and changes the bicarbonate pool of the patient. As a consequence, an unknown fraction of the whole body bicarbonate flux is derived from the dialysis fluid (4). In the approach of Van Goudoever \textit{et al.} whole body bicarbonate flux is estimated prior to the $^{13}$C-valine infusion using a primed continuous infusion of NaH$^{13}$CO$_3$ of short duration. In this way, a 2 point calibration is obtained with background $^{13}$CO$_2$ enrichment at no infusion of NaH$^{13}$CO$_3$ and the measured value of enriched CO$_2$ at the applied continuous infusion rate of NaH$^{13}$CO$_3$. The $^{13}$C bicarbonate flux originating from the oxidation of $^{13}$C-valine was then calculated by linear interpolation of the measured $^{13}$CO$_2$ enrichment in expired air at steady state during $^{13}$C-valine infusion between the 2 points of the calibration. In other words, the ratio of enrichments of $^{13}$CO$_2$ in expired air during $^{13}$C-valine infusion over that during NaH$^{13}$CO$_3$ infusion is a reflection of the ratio between the rate of $^{13}$C-bicarbonate production originating from the oxidation of $^{13}$C-valine over the rate of continuous infusion of NaH$^{13}$CO$_3$. From the KIVA enrichment, which represents the intracellular dilution of valine, we calculated the amount of valine being oxidised to sustain this calculated production of $^{13}$C-bicarbonate. This results in the following calculations:

$$i_{bic}(V) = \frac{IECO_2(V)}{IECO_2(B)} \times i(b)$$  \hspace{1cm} eq(2)

In which $i_{bic}(V)$ is the $^{13}$C-bicarbonate production from $^{13}$C-valine during valine infusion, $IECO_2(B)$ is the isotopic enrichment in atom percent enrichment (APE) of $^{13}$CO$_2$ in expired air at isotopic steady state during the NaH$^{13}$CO$_3$ infusion, $IECO_2(V)$ is the isotopic enrichment in APE of $^{13}$CO$_2$ in expired air at isotopic steady state during the $^{13}$C-valine infusion, and $i(b)$ is the NaH$^{13}$CO$_3$ infusion rate in $\mu$mol kg$^{-1}$ h$^{-1}$. Valine oxidation was calculated according to:

$$Ox = i_{bic}(V) \times 5 \times \left(\frac{100}{MPE(KIVA)}\right) \text{ in } \mu\text{mol kg}^{-1} \text{ h}^{-1}$$  \hspace{1cm} eq(3)

Where 5 is the number of carbon atoms in valine. In this way, the oxidation rate of $^{13}$C-valine could be calculated without measuring Vco$_2$.

During fasting: $Ox = O(\text{fast})$  \hspace{1cm} eq(4)
During the meal period recovery of labelled CO$_2$ will be increased in comparison to fasting. Estimates from literature have been used, *i.e.* 0.74 ± 7 to 0.84 ± 8 during fasting and meal intake respectively (30-32). This represents an increase of approximately 13%. Correction of the rate of oxidation of valine during the meal period is necessary because the 2 point calibration was done while the patient was fasting. O(fed) was thus calculated according to:

$O(fed) = Ox / 1.13$  

\text{eq}(5)$

**Calculation of whole body protein metabolism**

In figure 2, the steady-state isotopic model for whole body valine metabolism is depicted in a schematic diagram. In this model, influx of valine comes from whole body protein breakdown (B), and when appropriate from meal intake (I). Valine leaves the plasma amino acid pool by whole body protein synthesis (S), oxidation (O) and when applicable, dialysis (D). The input fluxes in this model result in label dilution of infused $[1^{13}C]$-valine in plasma. These fluxes have to be differentiated from those which result in changes in size of the plasma amino acid pool. This is of particular importance for the calculation of the rate of appearance of valine into plasma in the experiments in which the influence...

**Figure 2.** The model of whole body protein metabolism. In dark, the situation during fasting is represented, while in grey the meal intake and pool enlargement ($\Delta Q$) are added to the scheme. The fluxes shown are whole body protein breakdown (B), synthesis (S), oxidation (O), dialysate loss (D), infusion of $^{13}C$-valine (i) and the dietary intake of valine (I), corrected for first pass absorption effects.
of protein intake has been studied. During protein intake plasma amino acid concentrations increased gradually. Therefore, the appearance of dietary valine into the circulation comprised a flux resulting in enlargement of the plasma valine pool, and a flux of dietary valine \( (I) \) adding to whole body protein metabolism. The appearance of dietary valine was multiplied by 0.8 to correct for first pass metabolism \((33;34)\). The amount of dietary valine entrapped in the enlarged pool size of valine \( (\Delta Q) \) was calculated by multiplying the increase in valine concentration in plasma by total body water defined as 60% of body weight in these patients \((35)\). The difference of plasma valine concentration before and at the end of the meal period was used to calculate the increase in the whole body valine pool. We observed that the increase of valine during dietary protein intake was continuous during our study period. We assumed this increase to be linear in time and the associated flux to be constant. Accordingly, the total rate of appearance of valine \( R(a) \) comprises appearance of valine released from breakdown \( (B) \), infusion of valine \( (i) \), and when appropriate protein intake \( (I) \). At steady state the rate of appearance of valine equals the rate of disappearance of valine \( R(d) \). The total rate of disappearance of valine comprises protein synthesis \( (S) \), protein oxidation \( (O) \), and when appropriate losses into the dialysate \( (D) \).

At steady state:

\[
R(a) = B + I + i = S + O + D = R(d) \text{ in } \mu\text{mol valine kg}^{-1} \text{ h}^{-1}
\]

This results in the following calculations:

Without dialysis during fasting \((\text{HD-fas})\), \( I = 0 \) and \( D = 0 \) so

\[
B = R(a) - i \quad \text{eq}(7)
\]

\[
S = R(a) - O(\text{fast}) \quad \text{eq}(8)
\]

Without dialysis during the meal period \((\text{HD-fed})\), \( I \neq 0 \) and \( D = 0 \) so

\[
B = R(a) - i - I \quad \text{eq}(9)
\]

\[
S = R(a) - O(\text{fed}) \quad \text{eq}(10)
\]

While dialysing during fasting \((\text{HD+fas})\), \( I = 0 \) and \( D \neq 0 \) so

\[
B = R(a) - i \quad \text{eq}(11)
\]

\[
S = R(a) - O(\text{fast}) - D \quad \text{eq}(12)
\]

While dialysing during the meal period \((\text{HD+fed})\), \( I \neq 0 \) and \( D \neq 0 \) so

\[
B = R(a) - i - I \quad \text{eq}(13)
\]

\[
S = R(a) - O(\text{fed}) - D \quad \text{eq}(14)
\]
### Table 1. Demographic and dialysis status of the studied chronic hemodialysis patients.

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<th>Weight (kg)</th>
<th>BMI (wt/height^2)</th>
<th>Albumin (G/l)</th>
<th>Kt/V</th>
<th>PI</th>
<th>EI (Kcal/kg/d)</th>
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<th>Weight (kg)</th>
<th>BMI (wt/height^2)</th>
<th>Albumin (G/l)</th>
<th>Kt/V</th>
<th>PI</th>
<th>EI (Kcal/kg/d)</th>
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<td>58</td>
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<td>31.4</td>
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<td>57/F</td>
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Kt/V, Measure of dialysis adequacy. PI, protein intake estimated from 1 week dietary recording. EI, energy intake from 1 week dietary recording. In italic, estimated from 1 day dietary recall by dietician instead of 1 week dietary recording. HD- patients measured during the non-dialysis protocol, HD+ patients measured during the dialysis protocol. There were no statistical differences between the two groups.

Protein balance was calculated by subtracting protein breakdown (B) from protein synthesis (S). Dialysate losses were calculated by measuring amino acid concentrations in spent dialysate in μmol l^-1 multiplied by the volume flow of dialysate in l h^-1.
Statistics
All values are given as means ± SD. Statistical analysis was done using SPSS 10.0 (SPSS Inc, Chicago, Illinois). To compare the changes in protein metabolism due to the meal, the fasting and fed state were compared using a paired student t-test. Differences between the protein metabolism parameters on a non-dialysis day and during dialysis were tested using the unpaired student t-test. Correlations between valine concentrations and protein metabolism parameters were tested using linear regression analysis and expressed using the Pearson Correlation coefficient. Statistical significance was assumed when p<0.05.

Results
Demographic data
Table 1 shows the demographic and clinical data of the patients studied. All patients were well nourished as can be concluded from the protein intake and serum albumin concentrations. Dialysis was adequate as shown by the equilibrated Kt/V values. There were no statistical differences between the two studied groups with respect to BMI, age, or albumin concentration. There were episodes of hypotension in 2 out of 6 patients only during dialysis with feeding, which could be reversed by discontinuing the ultrafiltration. The difference between the two dialysate glucose concentrations did not influence plasma glucose and insulin concentrations. Pre-dialysis glucose concentrations in plasma were 5.6 mM (range 3.9 - 7.5 mM) during fasting and did not change during the dialysis session. During the meal period, glucose concentrations in plasma were 6.8 mM (range 5.2 - 8.7 mM). Pre-dialysis insulin values in plasma were 11.6 mIU l\(^{-1}\) (range 6.1 - 15.3 mIU l\(^{-1}\)) during fasting and did not change during the dialysis session. During the meal period, insulin concentrations in plasma were 44.7 mIU l\(^{-1}\) (range 19.8 - 84.6 mIU l\(^{-1}\)). Glucose or insulin values did not correlate with the other studied variables in our subjects.

Amino acid concentrations
Losses of amino acids into the dialysate during the fasting period were 74 ± 21 mmol per patient per dialysis session (7.7 ± 2.1 g). The valine loss contributed 21 ± 4 μmol kg\(^{-1}\) h\(^{-1}\) to the total amino acid losses. The amino acid loss was 35 % higher during the study day with dialysis, while the patients consumed a meal, and was 105 ± 13 mmol per patient per session (11.7 ± 1.9 g). Valine losses contributed 35 ± 5 μmol valine kg\(^{-1}\) h\(^{-1}\) to this loss. Plasma valine concentration increased during dietary protein intake by 150 ± 31 μM on a non-dialysis day and 126 ±37 μM on a dialysis day. In figure 3 the arterial plasma AA concentrations are shown for each study group. Total AA, essential AA, and non-essential
AA were all significantly higher during feeding compared to fasting, both during the HD- protocol and during the HD+ protocol. Furthermore, there is a difference in response upon dialysis between the essential and non-essential amino acids. Whereas dialysis has only a minor, not significant, influence on the concentration of essential AA (HD- 844 μM to HD+ 732 μM) in plasma, the concentration of non-essential AA (HD- 2006 μM to HD+ 1269 μM) and thus total AA (HD- 2850 μM to HD+ 2001 μM) in plasma decreased significantly during the dialysis sessions during fasting. Consumption of a protein-enriched meal during the HD- protocol also changed the relative composition of plasma amino acids. Essential amino acids increased relatively more (844 μM to 1447 μM) than the non-essential amino acids (2006 μM to 2626 μM). Consumption of the meal during dialysis resulted in an increase of essential amino acids (732 μM to 1273 μM) and of non-essential amino acids (1269 μM to 1723 μM). It can be seen in figure 3 that the concentration of non-essential amino acids during dialysis and meal intake was lower than the non-essential amino acid concentration during the HD- protocol while fasted.

Figure 3. Concentrations of total (TAA), essential (EAA) and non-essential (NEAA) amino acids in plasma in the steady state periods in all study protocols. HD-fas is the study period during the non-dialysis day while fasting, HD-fed is the study period during the non-dialysis day with meal intake, HD+fas is the study period during a dialysis session while fasting, HD+fed is the study period during a dialysis session with meal intake. +: denotes significant difference in amino acid concentration during fasting compared to feeding. *: denotes significant difference in amino acid concentration during the HD+ protocol compared to the same condition (fasting or feeding) during the HD- protocol.
Protein metabolism

In figure 4, plateau enrichments for breath CO$_2$ enrichment and plasma KIVA enrichment are shown to illustrate their steady state in time. Figure 4A shows during the HD- protocol, plateau $^{13}$CO$_2$ enrichment in expired breath prior to the start of the experiment, during the NaH$^{13}$CO$_3$ infusion from 0.5 to 1 hour, and during the $^{13}$C-valine infusion while fasting (between 4 and 5 h.) or consuming a protein-enriched meal (between 7 and 8 h.). Figure 4B shows plateau enrichments during the HD- protocol of plasma KIVA during fasting (between 4 and 5 h.) and during consumption of a protein-enriched meal (between 7 and 8 h.). Figure 4C shows the plateau enrichments of $^{13}$CO$_2$ in expired air during dialysis, in the absence of any infusion of NaH$^{13}$CO$_3$ or $^{13}$C-valine. Furthermore, the time-course is shown of $^{13}$CO$_2$ in expired air during dialysis, in the absence of any infusion of NaH$^{13}$CO$_3$ or $^{13}$C-valine. Figure 4D shows the plateau enrichments during the HD+ protocol of plasma KIVA during fasting and feeding (between 3 and 4 h.). The effect of dialysis on total bicarbonate turnover is already clear from a
Table 2  Values of valine fluxes obtained by evaluation of primary data.

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<tr>
<th>Patients</th>
<th>Fluxes (µmol val kg(^{-1}) h(^{-1}))</th>
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<tr>
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<td>25 ± 7</td>
<td>117 ± 12</td>
<td>18 ± 4</td>
<td>35 ± 5</td>
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R(a) is the total rate of appearance of valine, O(fast) and O(fed) are the oxidation rates during fasting and consuming a protein-enriched meal, respectively, D is the dialysis loss, I is the appearance of dietary valine estimated from the intake of valine corrected for first pass absorption, J(ΔQ) is the flux of valine associated with the enlargement of the plasma valine pool.
comparison of steady state enrichments of $^{13}$CO$_2$ in expired air during NaH$^{13}$CO$_3$ isotope infusion in the HD- protocol ($\delta = +20 \pm 4$ ‰) and HD+ protocol ($\delta = +9 \pm 3$ ‰) due to the exchange of plasma bicarbonate for dialysate bicarbonate. In table 2 the summary is given of the evaluation of primary isotopic data and changes in valine concentration for the individual patients. As is clear from this table in fasting CHD patients the total rate of appearance of valine in plasma is not significantly influenced by dialysis ($77 \pm 8 \mu$mol kg$^{-1}$ h$^{-1}$ versus $80 \pm 15 \mu$mol kg$^{-1}$ h$^{-1}$). A similar observation can be made for the total rate of appearance of valine in plasma during dietary protein intake ($108 \pm 13 \mu$mol kg$^{-1}$ h$^{-1}$ versus $117 \pm 20 \mu$mol kg$^{-1}$ h$^{-1}$). Furthermore, it can be seen that the increase in plasma valine pool size is significantly higher in the HD- protocol ($38 \pm 8 \mu$mol kg$^{-1}$ h$^{-1}$) compared to the HD+ protocol ($25 \pm 7 \mu$mol kg$^{-1}$ h$^{-1}$), most likely due to the loss of valine ($35 \pm 5 \mu$mol kg$^{-1}$ h$^{-1}$) during dialysis. In table 3, data on whole body protein metabolism are given that were derived from the data given in table 2. The rate of whole body protein synthesis and oxidation in the fasting state were significantly lower during dialysis compared to the rate calculated during the HD- protocol. During the meal period, the rate of whole body protein breakdown was reduced during both the HD- protocol as well as during

<table>
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<th>Whole body protein flux</th>
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<tr>
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<tr>
<td>HD-</td>
<td>70 ± 8*</td>
<td>45 ± 9</td>
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<tr>
<td>HD+</td>
<td>65 ± 15*</td>
<td>33 ± 16</td>
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<td>HD+</td>
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<td>HD+</td>
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<tr>
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<td>21 ± 4*</td>
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All values expressed as mean ± SD in $\mu$mol kg$^{-1}$ h$^{-1}$ $+ p < 0.05$ between HD- and HD+ $* p < 0.05$ between fasting and meal intake. $++ p = 0.06$
the HD+ protocol. The rates of protein synthesis and oxidation were reduced during the HD+ protocol compared to the HD- protocol. Protein balance during fasting, calculated as the difference between the rates of whole body protein synthesis and breakdown, was significantly higher in the HD- protocol (-8 ± 5 μmol kg⁻¹ h⁻¹) as compared to the balance during the HD+ protocol (-15 ± 4 μmol kg⁻¹ h⁻¹). The consumption of a protein-enriched meal improved protein balance significantly compared to fasting in both protocols. During the HD- protocol, protein balance increased to 32 ± 9 μmol kg⁻¹ h⁻¹ while during the HD+ protocol, protein balance increased to 31 ± 5 μmol kg⁻¹ h⁻¹ (NS). Figure 5 illustrates whole body protein synthesis, breakdown, and balance during the fasting and meal period in the HD- and HD+ protocol. In the absence of dialysis, protein balance increased 39 ± 9 μmol kg⁻¹ h⁻¹ during the meal period while the increase during dialysis was 45 ± 4 μmol kg⁻¹ h⁻¹.

**Correlations**

Protein synthesis was positively correlated (Pearson r = 0.50, p<0.01) and protein breakdown negatively correlated (Pearson r = -0.54, p<0.01) with plasma valine concentration, tested using both parameters as continuous variables. In figure 6 this is illustrated for the four conditions separately.
The aim of the study was to test the hypothesis that consumption of a protein and energy enriched meal restores whole body protein balance during dialysis. Therefore we examined the effects of such a meal on whole body protein metabolism in CHD patients on a day between two dialysis days and during dialysis. We used a primed continuous infusion of $^{13}$C-valine and measurement of isotope dilution of $^{13}$C-KIVA in plasma and $^{13}$CO$_2$ in expired air. Our study shows that on a non-dialysis day protein balance was negative after an overnight fast. Consumption of a protein-enriched meal resulted in a positive whole body protein balance. During dialysis, fasting patients were in an even more negative protein balance than on a non-dialysis day. Consumption of a protein and energy enriched meal during dialysis resulted in a positive protein balance to the same extent as on a non-dialysis day. Dialysis led to considerable losses of plasma amino acids into dialysate, which could be supplemented by dietary amino acids but with a shift in composition between essential and non-essential amino acids.

Before interpreting our results, we would like to discuss some methodological issues. In

Discussion

The aim of the study was to test the hypothesis that consumption of a protein and energy enriched meal restores whole body protein balance during dialysis. Therefore we examined the effects of such a meal on whole body protein metabolism in CHD patients on a day between two dialysis days and during dialysis. We used a primed continuous infusion of $^{13}$C-valine and measurement of isotope dilution of $^{13}$C-KIVA in plasma and $^{13}$CO$_2$ in expired air. Our study shows that on a non-dialysis day protein balance was negative after an overnight fast. Consumption of a protein-enriched meal resulted in a positive whole body protein balance. During dialysis, fasting patients were in an even more negative protein balance than on a non-dialysis day. Consumption of a protein and energy enriched meal during dialysis resulted in a positive protein balance to the same extent as on a non-dialysis day. Dialysis led to considerable losses of plasma amino acids into dialysate, which could be supplemented by dietary amino acids but with a shift in composition between essential and non-essential amino acids.

Before interpreting our results, we would like to discuss some methodological issues. In
our study we used high infusion rates of $^{13}$C-valine during dialysis to measure enrichment of $^{13}$CO$_2$ in expired air with sufficient precision above background. Particularly, we anticipated low values of $^{13}$CO$_2$ enrichment in expired air in patients consuming a protein-enriched meal during a dialysis session. Accordingly, in the HD+ protocol the rate of infusion of $^{13}$C-valine was doubled compared to the HD- protocol. Under conditions of dialysis and dietary protein intake, $^{13}$CO$_2$ enrichment in expired air was low because, (i) isotope dilution of $^{13}$C-valine was considerable due to appearance of dietary valine and (ii) total bicarbonate production increased due to exchange of plasma bicarbonate with extracorporeal bicarbonate in dialysate. Enrichment of plasma $^{13}$C-KIVA during dialysis in the absence of protein intake was around 20%. Infusion of $^{13}$C-valine at such high rates is considered a “flooding” dose of tracer, which could perturb the processes to be studied (36-38). However, these perturbations will most likely be limited in the case of valine. Several studies have shown that a “flooding” dose of valine does not elicit an anabolic response of whole body protein metabolism (39;40). Furthermore, infusion of leucine affects plasma valine concentration, whereas infusion of valine does not affect plasma leucine concentration. (15;41). During the meal period, plasma $^{13}$C-KIVA enrichment decreased to values around 15%.

We used an independent infusion of NaH$^{13}$CO$_3$ of short duration to estimate whole body bicarbonate production, instead of indirect calorimetry. During dialysis, plasma bicarbonate exchanges with extra-corporeal bicarbonate in dialysate. With indirect calorimetry this effect of dialysis on the whole body bicarbonate content can not be estimated, since this method measures the net effect of this exchange. Accordingly, the dilution of $^{13}$CO$_2$ derived from $^{13}$C-valine oxidation in the body bicarbonate pool can not be determined accurately, by applying Vco$_2$ measured by indirect calorimetry while the patient was dialysing. In one study using indirect calorimetry to measure whole body bicarbonate production, the bicarbonate influx from the dialysis machine was estimated by taking the arterial-venous difference in bicarbonate concentration across the dialysis machine (4). Influx of bicarbonate from the dialysis machine was calculated to be negligible compared to whole body bicarbonate flux. This is true for net bicarbonate gain during the dialysis procedure. However, arterial-venous differences do not measure the unidirectional fluxes of bicarbonate exchange across the dialysing membrane and this is what matters in isotope dilution studies. These unidirectional fluxes contribute to the apparent increase in whole body bicarbonate production, observed as the increase of isotope dilution of CO$_2$ due to dialysis ($\delta \approx +10\%$) compared to non-dialysis ($\delta \approx +20\%$). Additionally, bicarbonate dissolved in dialysate was found to be naturally enriched ($\delta \approx -4\%$) in comparison with background enrichment of plasma bicarbonate in our patients ($\delta \approx -25\%$). Exchange of
bicarbonate between plasma and dialysate resulted in a gradual increase in \(^{13}\text{CO}_2\) background enrichment which reached steady-state in the last 3 hours of dialysis (\(\delta \approx -20\%\)). We corrected for these changes in \(^{13}\text{CO}_2\) background enrichment, otherwise oxidation rates would have been overestimated by almost 20%. This overestimation of the oxidation rate would have resulted in an underestimation of protein synthesis. The significant changes in \(^{13}\text{CO}_2\) background enrichment observed in our studies precluded comparison of whole body protein metabolism immediately preceding the dialysis session with that during dialysis and immediately after dialysis in a single measurement.

Turning to our results, we found that dialysis mainly decreased whole body protein synthesis and to a lesser extent whole body protein oxidation. Whole body protein breakdown was not significantly affected or, in other words, the rate of appearance of valine in plasma, corrected for infusion of labelled valine, was not affected by dialysis. In several studies a similar observation was made (4;42). However, Ikizler \textit{et al.} (7) showed an increase in protein breakdown upon dialysis. Although the reason for this discrepancy is not clear, there are differences in the execution of the studies in comparison with our study. Leucine oxidation rates were estimated from the appearance of \(^{13}\text{CO}_2\) in expired air and the total \(\text{CO}_2\) production measured by indirect calorimetry. Only Lim \textit{et al.} corrected for the loss of \(^{13}\text{CO}_2\) into dialysate, albeit with a value based on theoretical considerations. We measured the bicarbonate flux in each patient studied while dialysing. We extended the isotopic model of whole body protein metabolism to accommodate dietary valine influx and losses of valine into dialysate. When this model is applied to the results of our measurements in fasted, non-dialysing CHD patients, interpretation is straightforward. In this case, appearance of valine in plasma, corrected for infusion of isotope, arises from endogenous sources, i.e. whole body protein breakdown, and whole body protein synthesis equals non-oxidative disposal of valine. In cases of protein intake and/or dialysis the model becomes more complicated. We reasoned that during dialysis loss of valine in dialysate contributed to the non-oxidative disposal of valine. Accordingly, the associated flux was subtracted from the rate of non-oxidative disposal of valine. Ikizler \textit{et al.} (7) used another modelling approach for the amino acid losses, which might have influenced their conclusion.

Consumption of a protein-enriched meal by CHD patients on a non-dialysis day resulted in a positive whole body protein balance. Whole body protein breakdown was reduced to about two third the rate observed during fasting in these patients. Synthesis was slightly increased to 125% and oxidation was strongly increased to 205%. In view of the absolute values of the rates of whole body protein breakdown, the positive whole
body protein balance at the end of a meal was mainly the consequence of the strong inhibition of whole body protein breakdown. Similar observations have been made in apparently healthy individuals (11;12). A difference with earlier studies is that we corrected for the enlarged valine pool. During the consumption of the protein-enriched meal, valine concentration in plasma of CHD patients increased continuously. Accordingly, dietary valine influx was calculated as the difference between the enteral release of valine appearing into the circulation and the flux of valine associated with the enlargement of the plasma valine pool (see figure 2). This correction of the enteral release of valine for pool enlargement makes the calculation of whole body protein breakdown sensitive to changes in the size of the plasma valine pool. It does not influence the calculation of whole body protein synthesis. Furthermore, we assumed that enteral release of valine was the same as the amount of ingested valine hydrolysed in 0.5 h and that first pass absorption was 20 % (33;34). This represents, most likely, an oversimplification but it will not change the conclusions drawn in this paper. When different values for this first pass effects are brought into the calculations, whole body protein breakdown will increase proportionally in both HD-fed and HD+fed protocols.

Protein intake by CHD patients during dialysis restored the whole protein balance completely, when compared to a non-dialysis day. The effect of dietary protein intake on whole body protein synthesis and oxidation measured in the HD+ protocol were comparable to the HD- protocol, i.e. an increase to 128 % and 200 % of fasting values respectively, as shown in table 3. Furthermore, the effect of protein intake was comparable between the HD+ and HD- protocol with respect to the rate of appearance of valine, corrected for the infusion of labelled valine. Protein breakdown was reduced to about half the rate observed during fasting in these patients. It might well be that the high effectiveness of dietary protein in inhibiting whole body protein breakdown during dialysis might be overestimated because of the corrections used to account for the increase of the valine pool. The valine concentration in plasma during dialysis increased less than on a non-dialysis day. The associated flux of dietary valine to enlarge the plasma valine pool is thus smaller and the calculated value of whole body protein breakdown becomes larger. The values of whole body protein balance thus represents a minimal estimate under the condition of a patient during dialysis while consuming a protein-enriched meal.

Recently, Pupim et al. (43) published their study on the effect of parenteral nutrition during dialysis on whole body and forearm protein metabolism in CHD patients. Infusion of an amino acid solution, containing dextrose and lipids as well, during dialysis, resulted in an inhibition of whole body protein breakdown and stimulation of protein synthesis.
both by about 50%. Although qualitatively the same, quantitatively there are discrepancies with our study. As yet, we do not have an explanation. It might be the consequence of differences in experimental set-up or in the model used in the calculations. Pupim et al. applied an intravenous infusion of amino acids, together with dextrose and lipids, whereas we used a protein and fat enriched meal. Similar to our observations during consumption of a protein-enriched meal, Pupim et al. observed an increase in the plasma amino acid concentrations in CHD patients during dialysis as a consequence of parenteral nutrition. It is not clear from their description of the isotopic model how they corrected for this increase in pool size.

Substantial amounts of plasma amino acids were lost during dialysis. Losses of amino acids in dialysate amounted to 7.7 ± 2.1 g of amino acids during dialysis of fasting patients, similar to published figures (44-46). Losses of amino acids were 11.7 ± 1.9 g in patients while consuming a protein-enriched meal. Similar losses were observed by Wolfson et al. (44) during their infusion of 39.5 g of amino acids with 200 g of glucose. Essential amino acid concentrations responded differently during dialysis than non-essential amino acid concentrations. When fasted patients were dialysed, plasma essential amino acids concentration decreased 13 % in comparison to the concentration on a non-dialysis day. The decrease in concentration of plasma non-essential amino acids was more pronounced (37 %). Since body protein is enriched in essential amino acids in comparison with plasma amino acids, breakdown of body protein will result in an increase of essential amino acids relative to non-essential amino acids in plasma as was shown in figure 3. Consumption of a protein-enriched meal on a non-dialysis day also changed the relative composition of plasma amino acids. The increase in concentration of plasma essential amino acids (71 %) was more pronounced than of plasma non-essential amino acids (31 %). In view of the amino acid composition of milk proteins, enteral protein hydrolysis will release essential in relative excess to non-essential amino acids. Combining the effects of dialysis and protein-enriched meal resulted in a 57 % increase in plasma essential amino acid concentration and in a small increase of plasma non-essential amino acid (26 %) concentrations. Thus, at the end of the dialysis session during which the patients consumed a protein-enriched meal, non-essential amino acids were in shortage relative to essential amino acids. This effect has not been described before. It is tempting to speculate that a disbalance in plasma free amino acid composition after dialysis prevents whole body protein metabolism to revert quickly to its normal, pre-dialysis, condition. Hypothetically, this relatively small derangement in protein metabolism could contribute to malnutrition over longer periods of time.
Oral intradialytic nutrition by means of a protein-enriched meal appeared to be an effective treatment for dialysis-induced protein loss due to clearance of plasma amino acids by the dialysis machine. In the study protocol we used a protein intake of 0.6 g protein kg$^{-1}$, comparable to 50% of daily protein intake in this group of patients. We think that this amount of protein might be too much for the average dialysis patient during a 4 hour dialysis session. Pupim et al. infused 15 g amino acids, whereas we estimated a dietary amino acid influx into the circulation of 39 g, assuming 80% of all protein taken was digested during the dialysis session. The effects of smaller doses of oral protein on whole body protein metabolism in CHD patients during dialysis are unknown but our results show that an oral protein load during dialysis has a positive effect that is not less than that of the same load given without dialysis.

In conclusion, we found that consumption of a protein and energy enriched meal abolished the negative effect of dialysis on whole body protein balance. This offers a possibility for nutritional intervention in preventing protein-energy malnutrition. It also shows that, even though a meal during dialysis may increase the occurrence of hypotension, it is metabolically useful and should therefore be standard practice.

Acknowledgements

The authors appreciate the time from the patients and nursing staff of the Dialysis Centre Groningen. Part of this work was presented at the 34th annual meeting of the American Society of Nephrology, 2001. This work was supported by a grant from the Dutch Kidney Foundation (nr C 97-1694).
Reference list


