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

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Veelanan, Jorden M., Hermi A. Kingma, Theo S. Boer, Frans Stellarda, Dirk-Jan Reijngoud, and Roel M. Huisman. Protein intake during hemodialysis maintains a positive whole body protein balance in chronic hemodialysis patients. Am J Physiol Endocrinol Metab 284: E954–E965, 2003. First published January 21, 2003; 10.1152/ajpendo.00264.2002.—Protein energy malnutrition is present in 18 to 56% of hemodialysis patients. Because 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. Patients were studied on a single day between dialysis (HD—protocol) in the morning while fasting and in the afternoon while consuming six 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 1-[13C]leucine. During HD—feeding changed the negative whole body protein balance observed during fasting to 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 with whole body protein synthesis and inversely correlated with whole body protein breakdown. We conclude that the consumption of a protein- and energy-enriched meal by CHD patients while dialyzing can strongly improve whole body protein balance. The role of CHD itself on protein metabolism are limited. 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 with a nondialysis day regardless of daily protein intake (5, 23). Lim et al. (22) studied whole body protein metabolism by applying the [13C]leucine isotope dilution technique in fasting CHD patients during hemodialysis. They observed a reduction in whole body protein synthesis compared with the predialysis period, and this resulted in a doubling of the negative protein balance already present in fasting CHD patients. Furthermore, hemodialysis stimulates muscle protein losses compared with the predialysis period in fasting CHD patients (18).

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 Refs. 8 and 45). Particularly, amino acids in plasma are powerful modulators of protein metabolism, as a mixture or in conjunction with insulin (42). 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 (29, 30). 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 nondialysis 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 the following two questions more specifically: 1) to what extent does consumption of a protein- and energy-enriched meal result in a positive whole body protein balance in CHD patients, and 2) 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 nondialysis day, the second question during two dialysis sessions separated by 1 wk. Whole body protein metabolism was studied by applying stable isotope infusion techniques using $[^{13}C]${valine} has been used previously by our laboratory in the study of whole body protein metabolism and synthesis of several specific proteins (34) 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 (9, 12), 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 (38).

### SUBJECTS AND METHODS

#### Study Subjects

All nondiabetic, stable hemodialysis patients aged under 65 yr in the Dialysis Center Groningen were approached to participate in the two protocols of the present study, i.e., a nondialysis and a dialysis protocol. Twelve patients gave their permission, but only three or 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 nondialysis protocol, and three patients participated only in the dialysis protocol (Table 1). The medical ethics 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 3 mo before the study protocol and had been in dialysis for 6 mo or more. The diagnoses were chronic glomerulonephritis in three patients (1 with hypertension), nephropathy resulting from hypertension in three patients, quiescent Wegeners disease in one patient, and polycystic kidney disease in three patients, and the cause of renal failure was unknown in two cases. Medications included phosphate binders, iron, multivitamins, antihypertensive drugs, calcitriol, and recombinant human erythropoietin of which the dose had not been altered for 3 mo before the study protocol to avoid altered hematopoiesis. No patients received steroid hormones or immunosuppressive agents in the 6 mo before the study protocol. The patients were dialyzed with low-flux biocompatible dialyzers for 4 h three times weekly. Blood flow ranged from 250 to 350 ml/min, and dialysate flow was 500 ml/min. Standard dialysate with 140 meq Na⁺, and 34 meq bicarbonate, was used for all patients. Glucose content in dialysate was 5.6 mM in two patients and 11.2 mM in four patients during their experimental dialysis sessions. Residual renal function was 3 ml/min or less, which corresponded to a dialysis adequacy (Kt/V) value of 0.45 wk⁻¹ or less.

#### Materials

L-$[^{13}C]${valine} and NaH$^{13}$CO₃, both with an enrichment of >99 atom percent excess, were purchased from Cambridge Isotope Laboratories (Andover, MA). 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 g yogurt (5.7 g protein, 7.4 g carbohydrate, and 5.4 g fat; Domo), 20 g cream (0.5 g protein, 0.7 g carbohydrate, and 6.3 g fat; Friesche vlag, Ede, the Netherlands), and 5 g protein-enriched milk powder (1.5 g protein, 2.4 g carbohydrate, and 0.8 g fat; Fortify, Nutricia). Consumption of a meal portion every 30 min for 3 h resulted in a dietary valine intake of $132 \pm 20 \mu$mol·kg⁻¹·h⁻¹ (37) and a fluid intake of 350 ml/h. The energy content of a meal portion was 386 kcal.

#### Table 1. Demographic and dialysis status of the studied chronic hemodialysis patients

![Table 1](image-url)
kcal/h. Meals were designed to give >50% of daily protein intake, 0.62 ± 0.09 g/kg protein, and 15 ± 2 kcal/kg 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 3 mo before both protocols and were in a good nutritional state (41).

**Experimental Design**

**Pilot experiments.** Dialysis by itself was found to gradually increase the $^{13}$CO$_2$ enrichment in expired air because of the entrance of bicarbonate with a high natural enrichment from the dialysate (~4.0 ± 0.3‰ vs. Pee Dee Belemnite limestone). Therefore, background enrichment in expired breath was measured independently in five patients during a dialysis session before 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 $[^{13}]$bicarbonate or $[^{13}]$valine infusion for calculations of whole body protein metabolism. In a second pilot experiment, the extent to which the rate of $[^{13}]$valine infusion had to be increased during dialysis was tested. 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 the background enrichment that had already been changed by exchange of plasma and dialysate bicarbonate. Doubling the $[^{13}]$valine infusion rate appeared to be sufficient.

**Study protocols.** The present study comprised two protocols. In the nondialysis protocol (HD−), patients were studied on a day between two dialysis days. Fasting whole body protein metabolism was measured in the morning after an overnight fast (HD− fas). On the same study day, in the afternoon, this was followed by the measurement of whole body protein metabolism while patients were consuming the meal (HD− fed). The dialysis protocol (HD+) could not be done on a single day and therefore consisted of two study days 1 wk apart. Patients were dialyzed 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. Before the study (3 wk), all patients visited the Dialysis Center Groningen for a dietary interview and instructions on dietary recording. Patients consumed a protein intake of 1.0 ± 0.1 g·kg$^{-1}$·day$^{-1}$, while caloric intake was not restricted.

**Nondialysis protocol.** In the HD− protocol, patients had fasted overnight and were studied during a midweek day without dialysis, having dialyzed the afternoon before. Patients were admitted to the Hospital Research Unit at ~7:30 AM. A catheter was inserted in 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 Fig. 1A. The NaH$^{13}$CO$_3$ infusion was started at 8:00 AM. During the 1st h, whole body bicarbonate production (details explained in Evaluation of Primary Data) was measured using a primed constant infusion of NaH$^{13}$CO$_3$ (5 μmol/kg bolus followed by a continuous infusion of 5 μmol·kg$^{-1}$·h$^{-1}$). Four breath samples were taken from 30 to 60 min after the start of the NaH$^{13}$CO$_3$ infusion at 10-min intervals. The NaH$^{13}$CO$_3$ infusion was discontinued immediately after the last breath sample was taken, and the L-$[^{13}]$valine infusion was started with a bolus of 15 μmol/kg followed by a continuous infusion of 7.5 μmol·kg$^{-1}$·h$^{-1}$ for the next 4 h. A second catheter was then inserted in the contralateral arm to collect blood samples. Blood and breath samples were taken simultaneously every half hour for 3 h after the start of the $[^{13}]$valine infusion. During the 4th h, blood and breath samples were taken every 15 min. At 1:00 PM, the meal period was started by consumption of the first portion of the protein-enriched meal and continued for 3 h by consumption of a portion every 30 min. $[^{13}]$Valine infusion continued at the same rate during this study period. Blood and breath samples were taken every 30 min for 2 h after the start of the meal, whereas during the last hour, samples were taken every 15 min. The study day ended at 4:00 PM. All catheters were removed, and patients were observed until stable and then discharged.

**Dialysis protocol.** In this protocol, patients were studied on two separate dialysis sessions, separated by 1 wk. On one occasion, patients were studied while they remained fasting.

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![Fig. 1. A: protocol used to study whole body protein metabolism in chronic hemodialysis (CHD) patients during a nondialysis day (HD−). After an overnight fast, whole body protein metabolism (HD− fas) was measured in the morning while patients fasted. 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). B: protocol used to study whole body protein metabolism in fasting CHD patients while dialyzing (HD+ fas). C: protocol used to study whole body protein metabolism in CHD patients consuming a protein- and energy-enriched meal during dialysis (HD+ fed).](http://ajpendo.physiology.org/DownloadedFrom)
On the second occasion, patients consumed six small meals, the first 1 h after the start of dialysis followed by five meals spaced by 30 min. Patients had been dialyzed 44 ± 3 h 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 center at ∼11:30 AM. Dialysis needles were inserted in the arterial-venous shunt to collect baseline blood samples, and breath samples were collected simultaneously. Dialysis started at ∼12:00 PM. A primed continuous infusion of NaH13CO3 was administered for 1 h through the venous line of the dialysis machine (5 μmol/kg bolus followed by a continuous infusion of 5 μmol·kg−1·h−1), and four breath samples were taken from 30 to 60 min after the start of the infusion at 10-min intervals. The NaH13CO3 infusion was discontinued after the last breath sample was taken, and a primed continuous infusion of L-[1-13C]valine was started through the venous line of the dialysis machine for 3 h (15 μmol·kg−1·h−1). Blood samples from the arterial line of the dialysis machine and breath samples were taken every half hour for the first 2 h after the start of the [13C]valine infusion. During the third and last hour, blood and breath were sampled every 15 min (Fig. 1B). When whole body protein metabolism was studied during the meal period, the same experimental setup was used as described above, with the exception that, at the start of the [13C]valine infusion, the first of the six meal portions was consumed, whereas the remaining five were consumed every 30 min during the next 3 h (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 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. Blood (4 ml) was drawn for each sample in liquid-heparinized vacuum tubes and centrifuged at 3,000 rpm. Plasma was extracted and stored at −20°C until analysis. Breath samples were collected in gas collection tubes with a straw, as described earlier (43). Subjects exhaled normally through a straw in 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 was stored at −20°C until analysis.

Amino acid concentrations in plasma and spent dialysate were measured by the AccQ Tag method using HPLC according to the manufacturer’s protocols (Waters, Breda, The Netherlands). Amino acids were grouped according to total amino acids, essential amino acids (the sum of the concentration of all individual amino acids, essential amino acids (the sum of the concentration of arginine, histidine, lysine, methionine, phenylalanine, threonine, isoleucine, leucine, and valine), and the nonessential amino acids (the concentration of total amino acids − essential amino acids). Insulin in plasma was determined by a double AB system, as described earlier (24). Glucose and albumin concentrations were determined by standard clinical chemistry methods.

Measurement of 13CO2 isotopic enrichment was performed by sampling directly the glass container with a Finnigan TracerMat (Finnigan MAT, San Jose, CA) continuous-flow isotope ratio-mass spectrometer as described by Vonk et al. (43).

The determination of L-[1-13C]valine isotopic enrichment was done as described earlier (32). In short, amino acids were isolated from deproteinized plasma using a cation exchange column (SCX-100, 209800; Alltech, Deerfield, IL). The isolated amino acids were derivatized to their corresponding N(O)-methoxy carbonyl methyl ester (MCM) according to Husek (15). Analysis of isotopic enrichment of plasma [13C]valine was carried out by GC-MS on a Hewlett Packard 5890 Plus gas chromatograph coupled to a Finnigan SSQ 7000 quadrupole mass spectrometer using positive-ion chemical ionization. The gas chromatograph was fitted with a capillary column (AT 1701; Alltech). The mass spectrometer was operated in the selected ion-monitoring mode at fragments with a mass-to-charge ratio (m/z) 190/191 of the [MH]+ and [MH+1]+ ions of the MCM derivative of unlabeled valine and L-[1-13C]valine, respectively.

α-[1-13C]ketoisovaleric acid (KIVA) isotopic enrichment was determined according to Kulik et al. (20). In short, standards with a tracer mole ratio for [1-13C]KIVA ranging from 0 to 22% were prepared by enzymatic conversion of standards of [1-13C]valine with the corresponding tracer mole ratio, as described earlier (33). Standards of [1-13C]KIVA and patient plasma samples were processed in the same series. KIVA was converted to 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 quadrupole mass spectrometer using positive-ion electron-impact ionization. The gas chromatograph was fitted with a capillary column (AT 1701; Alltech). The mass spectrometer was operated in the selected ion-monitoring mode recording fragments at m/z 245 and 246 of unlabeled KIVA and [1-13C]KIVA, respectively. All isotopic enrichments were measured against standard calibration curves.

Evaluation of Primary Data

Rate of appearance of intracellular valine (Ra) was calculated at isotopic steady state using the inverted pool model described by Matthews and colleagues (26–28) for leucine kinetics. When this isotopic model is applied to [1-13C]valine, enrichment of plasma KIVA is assumed to provide an estimate of intracellular enrichment of valine. The rate of appearance (μmol valine·kg−1·h−1) was calculated according to

\[ R_a = \frac{\text{MPE}(\text{V})}{\text{MPE}(\text{KIVA})} - 1 \times \frac{i(\text{V})}{i(\text{KIVA})} \]

where MPE(V) is the isotopic enrichment of the valine in the infusionate in mole percent excess, MPE(KIVA) is the isotopic enrichment of KIVA in plasma in mole percent excess, and i(\text{V}) is the infusion rate of [1-13C]valine (μmol·kg−1·h−1).

The rate of oxidation of valine was calculated following the approach described by Van Goudoever et al. (40). We did not use indirect calorimetry in our study to determine CO2 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 (22). In the approach of Van Goudoever et al., whole body bicarbonate flux is estimated before the [13C]valine infusion using a primed continuous infusion of NaH13CO3 of short duration. In this way, a two-point calibration is obtained with background 13C enrichment at no infusion of NaH13CO3 and the measured value of enriched CO2 at the applied continuous infusion rate of NaH13CO3. The [13C]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 two 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 oxidized to sustain this calculated production of $^{13}$C bicarbonate. This results in the following calculations

$$i_{\text{int}}(V) = \left[ \frac{\text{IECO}_2(V)}{\text{IECO}_2(B)} \right] \times i(b)$$

in which $i_{\text{int}}(V)$ is the $^{13}$C bicarbonate production from $^{13}$C]valine during valine infusion, $\text{IECO}_2(B)$ 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, $\text{IECO}_2(V)$ 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 micromoles per kilogram per hour. Valine oxidation (Ox) was calculated according to

$$\text{Ox} = \frac{\text{I} \times \text{IECO}_2(V) \times 5 \times [100/MPE(KIVA)]}{\text{mol of -kg}^{-1} \text{h}^{-1}} \times i(b)$$

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(fast).

During the meal period, recovery of labeled CO$_2$ will be increased in comparison with fasting. Estimates from the literature have been used, i.e., 0.74 ± 7 to 0.84 ± 8 during fasting and meal intake, respectively (11, 14, 21). This represents an increase of ~13%. Correction of the rate of oxidation of valine during the meal period is necessary because the two-point calibration was done while the patient was fasting. O(fed) was thus calculated according to

$$\text{O(fed)} = \frac{\text{Ox}}{1.13}$$

**Calculation of Whole Body Protein Metabolism**

In Fig. 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 $^{13}$C]valine in plasma. These fluxes have to be differentiated from those that 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 in plasma in the experiments in which the influence of protein intake has been studied. During protein intake, plasma amino acid concentrations increased gradually. Therefore, the appearance of dietary valine in the circulation comprised a flux resulting in enlargement of the plasma valine pool and a flux of dietary valine, adding to whole body protein metabolism. The appearance of dietary valine was multiplied by 0.8 to correct for first-pass metabolism (10, 13). 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 (6). 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 comprises the appearance of valine released from breakdown, infusion of valine (i), and, when appropriate, protein intake. At steady state, the rate of appearance of valine equals the rate of disappearance of valine. The total rate of disappearance of valine comprises protein synthesis, protein oxidation, and, when appropriate, losses in the dialysate. At steady state

$$R_s = B + I + S + O + D = R_s (\text{pmol valine-kg}^{-1} \text{h}^{-1})$$

This results in the following calculations.

Without dialysis during fasting (HD− fas), I = 0 and D = 0; so

$$B = R_s - i$$

and

$$S = R_s - O(fast)$$

Without dialysis during the meal period (HD− fed), I ≠ 0 and D = 0; so

$$B = R_s - i - I$$

and

$$S = R_s - O(fed)$$

While dialyzing during fasting (HD + fas), I = 0 and D ≠ 0; so

$$B = R_s - i$$

and

$$S = R_s - O(fast) - D$$

While dialyzing during the meal period (HD + fed), I ≠ 0 and D ≠ 0; so

$$B = R_s - i - I$$

and

$$S = R_s - O(fed) - D$$

Protein balance was calculated by subtracting protein breakdown from protein synthesis. Dialysate losses were calculated by measuring amino acid concentrations in spent dia-
lysate in micromoles per liter multiplied by the volume flow of dialysate in liters per hour.

Statistics
All values are given as means ± SD. Statistical analysis was done using SPSS 10.0 (SPSS, Chicago, IL). To compare the changes in protein metabolism resulting from the meal, the fasting and fed states were compared using a paired Student’s t-test. Differences between the protein metabolism parameters on a nondialysis day and during dialysis were tested using the unpaired Student’s 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 at 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 study groups with respect to body mass index, age, or albumin concentration. There were episodes of hypotension in two out of six 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. Predialysis 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). Predialysis insulin values in plasma were 11.6 μIU/l (range 6.1–15.3 μIU/l) during fasting and did not change during the dialysis session. During the meal period, insulin concentrations in plasma were 44.7 μIU/l (range 19.8–84.6 μIU/l). Glucose or insulin values did not correlate with the other studied variables in our subjects.

Amino Acid Concentrations
Losses of amino acids in the dialysate during the fasting period were 74 ± 21 mmol-patient−1·dialysis session−1 (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-patient−1·session−1 (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 nondialysis day and 126 ± 37 μM on a dialysis day. In Fig. 3, the arterial plasma amino acid concentrations are shown for each study group. Total amino acids, essential amino acids, and nonessential amino acids were all significantly higher during feeding compared with fasting, both during the HD− protocol and during the HD+ protocol. Furthermore, there was a difference in response upon dialysis between the essential and nonessential amino acids. Although dialysis has only a minor, not significant, influence on the concentration of essential amino acids (HD − 844 μM to HD + 732 μM) in plasma, the concentration of nonessential amino acids (HD − 2,006 μM to HD + 1,269 μM) and thus total amino acids (HD − 2,850 μM to HD + 2,001 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 to 1,447 μM) than the nonessential amino acids (2,006 to 2,626 μM). Consumption of the meal during dialysis resulted in an increase of essential amino acids (732 to 1,273 μM) and of nonessential amino acids (1,289 to 1,723 μM). It can be seen in Fig. 3 that the concentration of nonessential amino acids during dialysis and meal intake was lower than the nonessential amino acid concentration during the HD− protocol while fasted.

Protein Metabolism
In Fig. 4, plateau enrichments for breath CO₂ enrichment and plasma KIVA enrichment are shown to illustrate their steady state in time. Figure 4A shows, during the HD− protocol, plateau ¹³CO₂ enrichment in expired breath before the start of the experiment, during the NaH¹³CO₃ infusion from 0.5 to 1 h, and during the [¹³C]valine infusion while fasting (between 4 and 5 h) or while consuming a protein-enriched meal (between 7 and 8 h). Figure 4B shows plateau enrichments of plasma KIVA during the HD− protocol 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 ¹⁸CO₂ in
expired air during the HD+ protocol before the start of the experiment, during NaH\(^{13}\)CO\(_3\) infusion (between 0.5 and 1 h), and during the \([^{13}\text{C}]\text{valine} \) infusion while fasted or fed (between 3 and 4 h). 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 \([^{13}\text{C}]\text{valine} \). Figure 4D shows the plateau enrichments of plasma KIVA during the HD+ protocol during fasting and feeding (between 3 and 4 h). The effect of dialysis on total bicarbonate turnover is already clear from a comparison of steady-state enrichments of \(^{13}\)CO\(_2\) in expired air during NaH\(^{13}\)CO\(_3\) isotope infusion in the HD− protocol (δ = +20 ± 4%) and HD+ protocol (δ = +9 ± 3%) because of 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 ± 8 vs. 80 ± 15 \(\mu\text{mol}\cdot\text{kg}^{-1}\cdot\text{h}^{-1}\)). A similar observation can be made for the total rate of appearance of valine in plasma during dietary protein intake (108 ± 13 vs. 117 ± 20 \(\mu\text{mol}\cdot\text{kg}^{-1}\cdot\text{h}^{-1}\)). Furthermore, it can be seen that the increase in plasma valine pool size is significantly higher in the HD− protocol (38 ± 8 \(\mu\text{mol}\cdot\text{kg}^{-1}\cdot\text{h}^{-1}\)) compared with the HD+ protocol (25 ± 7 \(\mu\text{mol}\cdot\text{kg}^{-1}\cdot\text{h}^{-1}\)), most likely because of the loss of valine (35 ± 5 \(\mu\text{mol}\cdot\text{kg}^{-1}\cdot\text{h}^{-1}\)) during dialysis. In Table 3, data on whole body protein metabolism are given that were derived from the data given in Table 2. The rates of whole body protein synthesis and oxidation in the fasting state were significantly lower during dialysis compared with 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 and the HD+ protocol. The rates of protein synthesis and oxidation were reduced during the HD+ protocol compared with 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 \(\mu\text{mol}\cdot\text{kg}^{-1}\cdot\text{h}^{-1}\)) compared with the balance during the HD+ protocol (−15 ± 4 \(\mu\text{mol}\cdot\text{kg}^{-1}\cdot\text{h}^{-1}\)). The consumption of a protein-enriched meal improved protein balance significantly compared with fasting in both protocols. During the HD− protocol, protein balance increased to 32 ± 9 \(\mu\text{mol}\cdot\text{kg}^{-1}\cdot\text{h}^{-1}\), while during the HD+ protocol, protein balance increased to 31 ± 5 \(\mu\text{mol}\cdot\text{kg}^{-1}\cdot\text{h}^{-1}\) (not significant). Figure 5 shows whole body protein synthesis, breakdown, and balance during the fasting and meal period in the HD− and HD+ protocols. In the absence of dialysis, protein balance increased 39 ± 9 \(\mu\text{mol}\cdot\text{kg}^{-1}\cdot\text{h}^{-1}\) during the meal period, whereas the increase during dialysis was 45 ± 4 \(\mu\text{mol}\cdot\text{kg}^{-1}\cdot\text{h}^{-1}\).

**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; this was tested using both parameters as continuous variables. In Fig. 6, this is illustrated for the four conditions separately.
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 nondialysis 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 nondialysis day. Consumption of a protein- and energy-enriched meal during dialysis resulted in a positive protein balance to the same extent as on a nondialysis day. Dialysis led to considerable losses of plasma amino acids in dialysate, which could be supplemented by dietary amino acids but with a shift in composition between essential and nonessential amino acids.

Table 2. Values of valine fluxes obtained by evaluation of primary data

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Fasting</th>
<th>Consuming protein-enriched meal</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$R_a$</td>
<td>$O_{(fast)}$</td>
</tr>
<tr>
<td>Nondialysis Protocol</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>81</td>
<td>15</td>
</tr>
<tr>
<td>2</td>
<td>88</td>
<td>21</td>
</tr>
<tr>
<td>3</td>
<td>74</td>
<td>18</td>
</tr>
<tr>
<td>4</td>
<td>85</td>
<td>10</td>
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<tr>
<td>5</td>
<td>69</td>
<td>10</td>
</tr>
<tr>
<td>6</td>
<td>81</td>
<td>22</td>
</tr>
<tr>
<td>7</td>
<td>68</td>
<td>16</td>
</tr>
<tr>
<td>8</td>
<td>81</td>
<td>10</td>
</tr>
<tr>
<td>9</td>
<td>68</td>
<td>12</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>77 ± 8</td>
<td>15 ± 5</td>
</tr>
</tbody>
</table>

Dialysis Protocol

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Fasting</th>
<th>Consuming protein-enriched meal</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>91</td>
<td>11</td>
</tr>
<tr>
<td>3</td>
<td>62</td>
<td>7</td>
</tr>
<tr>
<td>6</td>
<td>83</td>
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<tr>
<td>11</td>
<td>98</td>
<td>13</td>
</tr>
<tr>
<td>12</td>
<td>85</td>
<td>8</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>80 ± 15</td>
<td>9 ± 2</td>
</tr>
</tbody>
</table>

$R_a$, total rate of appearance of valine; $O_{(fast)}$ and $O_{(fed)}$, oxidation rates during fasting and consuming a protein-enriched meal, respectively; $D$, dialysis loss; $I$, appearance of dietary valine estimated from the intake of valine corrected for first-pass absorption; $J(\Delta Q)$, flux of valine associated with the enlargement of the plasma valine pool.

Table 3. Summary of fluxes relevant in whole body protein metabolism during both study protocols

<table>
<thead>
<tr>
<th>Whole Body Protein Flux</th>
<th>Fasting</th>
<th>Protein intake</th>
</tr>
</thead>
<tbody>
<tr>
<td>Breakdown</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HD−</td>
<td>70 ± 8*</td>
<td>45 ± 9</td>
</tr>
<tr>
<td>HD+</td>
<td>65 ± 15*</td>
<td>33 ± 16</td>
</tr>
<tr>
<td>Synthesis</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HD−</td>
<td>62 ± 8*</td>
<td>77 ± 11</td>
</tr>
<tr>
<td>HD+</td>
<td>50 ± 12*</td>
<td>64 ± 14‡</td>
</tr>
<tr>
<td>Oxidation</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HD−</td>
<td>15 ± 5*</td>
<td>31 ± 5</td>
</tr>
<tr>
<td>HD+</td>
<td>9 ± 2‡</td>
<td>18 ± 4‡</td>
</tr>
<tr>
<td>Valine loss in dialysate</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HD−</td>
<td>21 ± 4*</td>
<td>35 ± 5</td>
</tr>
<tr>
<td>HD+</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

All values expressed as means ± SD in μmol·kg$^{-1}$·h$^{-1}$. Valine losses in dialysate were calculated by multiplying the valine concentration in dialysate by the dialysate flow. $^*$P < 0.05 between fasting and meal intake. $^\dagger$P < 0.05 between HD− and HD+. $^\ddagger$P = 0.06.
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 with the HD− protocol. Under conditions of dialysis and dietary protein intake, \(^{13}\)CO\(_2\) enrichment in expired air was low because 1) isotope dilution of \(^{13}\)C-valine was considerable because of the appearance of dietary valine and 2) total bicarbonate production increased because of exchange of plasma bicarbonate with extracorporeal bicarbonate in dialysate. Enrichment of plasma \(^{13}\)C-KIVA during dialysis in the absence of protein intake was ~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 (25, 36, 44). 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 (7, 35). Furthermore, infusion of leucine affects plasma valine concentration, whereas infusion of valine does not affect plasma leucine concentration (1, 9). During the meal period, plasma \(^{13}\)C-KIVA enrichment decreased to values of ~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 extracorporeal bicarbonate in dialysate. With indirect calorimetry, this effect of dialysis on the whole body bicarbonate content cannot 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 cannot be determined accurately by applying \(V_{CO_2}\) measured by indirect calorimetry while the patient is dialyzing. 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 (22). Influx of bicarbonate from the dialysis machine was calculated to be negligible compared with 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 dialyzing 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\(_2\) resulting from dialysis (\(\delta\) approximately equal to \(+10\‰\)) compared with nondialysis (\(\delta\) approximately equal to \(+20\‰\)). Additionally, bicarbonate dissolved in dialysate was found to be naturally enriched (\(\delta\) approximately equal to \(-4\‰\)) compared with background enrichment of plasma bicarbonate in our patients (\(\delta\) approximately equal to \(-25\‰\)). Exchange of bicarbonate between plasma and dialysate resulted in a gradual increase in \(^{13}\)CO\(_2\) background enrichment that reached steady state in the last 3 h of dialysis (\(\delta\) approximately equal to \(-20\‰\)). We corrected for these changes in \(^{13}\)CO\(_2\) background enrichment, otherwise oxidation rates would have been overestimated by ~20%. This overestimation of the oxidation rate would have resulted in an underestimation of protein synthesis. The significant changes in \(^{13}\)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 labeled valine, was not affected by dialysis. In several studies a similar observation was made (3, 22). However, Ikizler et al. (18) showed an increase in protein breakdown upon dialysis. Although the reason for this discrepancy is not clear, there are differences in the execution of those studies compared with our study. Leucine oxidation rates were estimated from the appearance of \(^{13}\)CO\(_2\) in expired air and the total \(CO_2\) production measured by indirect calorimetry. Only Lim et al. (22) corrected for the loss of \(^{13}\)CO\(_2\) in dialysate, albeit with a value based on theoretical considerations. We measured the bicarbonate flux in each patient studied while dialyzing. We extended the isotopic model of whole body protein metabolism to accommodate dietary valine influx and losses of valine in dialysate. When this model is applied...
to the results of our measurements in fasted, nondialyzed 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 nonoxidative 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 nonoxidative disposal of valine. Accordingly, the associated flux was subtracted from the rate of nonoxidative disposal of valine. Ikizler et al. (18) used another modeling approach for the amino acid losses, which might have influenced their conclusion.

Consumption of a protein-enriched meal by CHD patients on a nondialysis day resulted in a positive whole body protein balance. Whole body protein breakdown was reduced to about two-thirds 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 (29, 30). 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 in the circulation and the flux of valine associated with the enlargement of the plasma valine pool (see Fig. 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 hydrolyzed in 0.5 h and that first-pass absorption was 20% (10, 13). This represents, most likely, an oversimplification, but it will not change the conclusions drawn in this study. When different values for the first-pass effects are brought into the calculations, whole body protein breakdown will increase proportionally in both HD− and HD+ protocols.

Protein intake by CHD patients during dialysis restored the whole protein balance completely compared with a nondialysis day. The effects of dietary protein intake on whole body protein synthesis and oxidation measured in the HD+ protocol were comparable to those in 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 labeled valine. Protein breakdown was reduced to about one-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 nondialysis 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 represent a minimal estimate under the condition of a patient during dialysis while consuming a protein-enriched meal.

Recently, Pupim et al. (31) 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 by ~50% each. 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 setup 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 (16, 39, 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. (46) during their infusion of 39.5 g of amino acids with 200 g of glucose. Essential amino acid concentrations responded differently during dialysis than nonessential amino acid concentrations. When fasted patients were dialyzed, plasma essential amino acid concentration decreased 13% compared with the concentration on a nondialysis day. The decrease in concentration of plasma nonessential amino acids was more pronounced (37%). Because body protein is enriched in essential amino acids compared with plasma amino acids, breakdown of body protein will result in an increase of essential amino acids relative to nonessential amino acids in plasma, as was shown in Fig. 3. Consumption of a protein-enriched meal on a nondialysis day also changed the relative composition of plasma amino acids. The increase in concentration of plasma essential amino acids (71%) was more pronounced than the increase of plasma nonessential amino acids (31%). In view of the amino acid composition of milk proteins, enteral protein hydrolysis will release essential amino acids in...
relative excess to nonessential amino acids. Combining the effects of dialysis and a protein-enriched meal resulted in a 57% increase in plasma essential amino acid concentration and in a small increase of plasma nonessential amino acid (26%) concentrations. Thus, at the end of the dialysis session during which the patients consumed a protein-enriched meal, nonessential amino acids were in shortage relative to essential amino acids. This effect has not been described before. It is tempting to speculate that a misbalance in plasma free amino acid composition after dialysis prevents whole body protein metabolism to revert quickly to its normal, predialysis, 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 resulting from clearance of plasma amino acids by the dialysis machine. In the study protocol, we used a protein intake of 0.6 g protein/kg, 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-h dialysis session. Pupim et al. (31) infused 15 g of amino acids, whereas we estimated a dietary amino acid influx in the circulation of 39 g, assuming that 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.

We appreciate the time from the patients and nursing staff of the Dialysis Center Groningen. Part of this work was presented at the 34th Annual Meeting of the American Society of Nephrology, San Francisco, CA, 2001. This work was supported by Grant no. C 97-1894 from the Dutch Kidney Foundation.

REFERENCES


