Whole body protein metabolism in chronic hemodialysis
Veeneman, Jorden Marcus

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The metabolic response to ingested protein is normal in long-term hemodialysis patients.
Abstract

**Background.** Protein-energy malnutrition affects 30-50% of hemodialysis (HD) patients. This has been attributed to an inadequate food intake but may be due to disturbances in utilisation of ingested protein.

**Methods.** We studied protein kinetics during fasting and during ingestion of a protein-enriched meal to investigate possible metabolic differences between stable HD patients and control subjects. Whole-body protein kinetics were measured by primed-constant infusion of L[1-$^{13}$C] valine.

**Results.** During fasting, whole body protein balance was significantly less negative in HD patients compared to control subjects. During meal intake, protein balance was similar between HD patients and control subjects. Meal intake increased protein balance significantly in both groups but not differently between the groups. Also, protein oxidation was decreased during fasting in HD patients compared to control subjects but not during meal intake.

**Conclusion.** We conclude that the rate of protein breakdown is lower in HD patients compared to control subjects but the efficiency of protein utilisation is normal in HD patients during a non-dialysis day.

**Keywords:** Valine turnover, protein synthesis, stable isotopes, amino acids, protein intake, hemodialysis patients.
Introduction

Signs of protein-energy malnutrition are frequently observed in patients with chronic renal failure (CRF) and remain present when hemodialysis therapy is started (1;2). This malnutrition is a major risk factor for increased morbidity and mortality in the maintenance hemodialysis (HD) patient (3). Nitrogen balance studies in HD patients have demonstrated that a neutral or even positive balance can be achieved at protein intakes of approximately 1.0 g kg\(^{-1}\) per day (4;5). However, nitrogen balance studies can not detect small disturbances in the dynamics of protein metabolism, which could explain the protein-energy malnutrition, when present over a long period of time.

Tracer methodology has been applied to study the dynamics of whole body protein kinetics by infusing amino acids labelled with stable isotopes. With this method, whole body protein breakdown, synthesis, and oxidation can be estimated separately. Applying this technique, Goodship et al. (6) compared whole body protein kinetics during fasting in pre-dialysis patients and healthy subjects after a 1-week dietary period during which the protein intake was 1.0 g kg\(^{-1}\) day\(^{-1}\). They did not observe significant differences in whole body protein kinetics in CRF patients compared to control subjects. Berkelhammer et al. (7) found that in fasting HD patients protein oxidation was increased, breakdown was not significantly changed and accordingly, synthesis was reduced when patients were compared to control subjects. Habitual protein intake was not commented on in this study. From these studies so far, no consistent picture of the cause of protein-energy malnutrition has emerged in CRF or HD patients when compared to control subjects.

The studies so far were performed in fasted hemodialysis patients. The importance of amino acids in plasma and insulin as powerful modulators of whole body protein balance has been well documented in apparently healthy volunteers (for a review see (8)). In control subjects, meal intake results in a reversion of the negative protein balance observed after an overnight fast (9;10). In this respect, protein-energy malnutrition in HD patients might be due to an impaired anabolic response to ingested protein during the non-dialysis time. In a previous publication, we studied the effects of dialysis on whole body protein kinetics in HD patients (11). In the present paper, we describe whether whole body protein metabolism in HD patients is different from healthy control subjects. More specifically we tried to answer the following questions, (i) how do whole body protein breakdown, synthesis, and oxidation in HD patients compare to breakdown, synthesis, and oxidation in control subjects during fasting or consumption of a protein enriched meal, and (ii) how
does the anabolic response in HD patients to a protein-enriched meal compare to the anabolic response in control subjects?

Subjects and Methods

Study subjects

Non-diabetic stable hemodialysis patients, aged less than 65 years, in the Dialysis Centre Groningen were requested to participate in the present study. Nine patients gave their permission (table 1). The patients in this study were the same as described in a previous study by our group (11). In addition, six control subjects were recruited from the staff of the University Hospital Groningen. The medical ethical committee from the University of Groningen approved all studies and written consent was obtained from all participants. All patients were clinically stable and without intercurrent acute illness in the three months before the study protocol and were in dialysis 6 months or more. Three patients were diagnosed as chronic glomerulonephritis (one with hypertension), 3 as nephropathy due to hypertension, 1 as quiescent Wegeners disease, and 2 had polycystic kidney disease. Medications included phosphate binders, iron, multivitamins, antihypertensive drugs, calcitriol, and in 7 patients recombinant human erythropoietin. No patients received hormone or immunosuppressive agents for 6 months before the day of the measurement. Patients were dialysed on low flux biocompatible dialyzers for 3.5 to 4 hours three times weekly. Blood flow ranged from 250 to 350 ml min$^{-1}$ and dialysate flow was 500 ml min$^{-1}$. Standard dialysate with 140 mEq Na$^+$, and 34 mEq bicarbonate was used for all patients. Glucose in dialysate was 5 mM. 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 valine intake of 90 ± 20 μmol kg$^{-1}$ h$^{-1}$ (12) and a fluid intake of 350 ml h$^{-1}$. Meals were designed to give at least 0.6 gram
kg\(^{-1}\) of protein and 15 kcal kg\(^{-1}\). It was assumed that gastric emptying during the meal was not influenced by dialysis in our HD patients since our patients had no history of dyspeptic symptoms in the three months before the study protocol started (13).

**Experimental design**

Three weeks prior to the study, all subjects visited the Dialysis Centre Groningen for a dietary interview and instructions on dietary recording. Patients consumed their regular protein intake, which was 1.0 ± 0.1 g kg\(^{-1}\) day\(^{-1}\) while control subjects adapted to this protein level. Patients were studied on a non-dialysis day between two dialysis days. Control subjects were studied during a midweek day. Subjects were fasted overnight and fasting whole body protein kinetics were measured during the morning. In the afternoon, this was followed by the measurement of fed whole body protein kinetics while subjects were consuming the protein-enriched meal. Subjects were admitted to the Hospital Research Unit at ≈ 7:30 AM. A catheter for IV infusions was inserted into the dorsal vein of the hand of the shunt arm of the HD patient or right arm of the control subjects and a second catheter was inserted in the contralateral arm to collect blood samples. Breath samples were taken simultaneously. A schematic diagram of the study day is shown in figure 1. The NaH\(^{13}\)CO\(_3\) 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\(^{13}\)CO\(_3\) (5 μmol kg\(^{-1}\) bolus followed by a continuous infusion of 5 μmol kg\(^{-1}\) h\(^{-1}\)). Four breath samples were taken every 10 minutes from 30 to 60 minutes after the start of the NaH\(^{13}\)CO\(_3\) infusion. The NaH\(^{13}\)CO\(_3\) infusion was discontinued immediately after the last breath sample was taken and the L[1-\(^{13}\)C]-valine infusion was started with a bolus of 15 μmol kg\(^{-1}\) followed by a continuous infusion of 7.5 μmol kg\(^{-1}\) h\(^{-1}\) for 4 hours. Blood and breath samples were taken simultaneously every 30 minutes for three hours after the start of the \(^{13}\)C-valine infusion. During the fourth hour, blood and breath samples were taken every 15 minutes. At 1:00 PM after the last samples were taken, the meal 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. The infusion of \(^{13}\)C-valine was continued at the same rate during this time. 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 and control subjects were observed until stable and then discharged.

**Analytical procedures**

Four ml of blood was drawn for each sample in liquid heparinized vacuum tubes and centrifuged at 3000 rotations per minute. Plasma was extracted and stored at –20 °C until analysis. Breath samples were collected in gas collection tubes using a straw as described earlier (14). 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. Amino acid concentrations in plasma were measured by AccQ Tag® method using high-performance liquid chromatography (HPLC) according to the manufacturers protocols (Waters BV. Breda, The Netherlands). Amino acids were grouped according to total amino acids (TAA), the sum of the concentration of all individual amino acids, essential amino acids (EAA, the sum of the concentration of arginine, histidine, lysine, methionine, phenylalanine, threonine, iso-leucine, leucine, and valine), and the non-essential amino acids (NEAA, the concentration of the TAA minus EAA).

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. (14).

The determination of L-[1-\(^{13}\)C] valine isotopic enrichment was done as described earlier (11). 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 (15). 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 $m/\nu$ 190/191 of the [MH]$^+$ and [MH+1]$^+$ ions of the MCM derivative of unlabelled and L-[1-$^{13}$C]-valine respectively.

$[1-^{13}$C]-KIVA (alpha-keto-isovaleric acid) was measured as its quinoxalinol-O-t-butyldimethylsilyl derivative. 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 (16). Standards of $[1-^{13}$C] KIVA and patient plasma samples were processed in the same series. 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 electron impact ionisation. The gas chromatograph was fitted with a capillary column (Alltech, AT 1701, length 20 m, id. 0.18 mm, film thickness 0.40 µm). The mass spectrometer was operated in the selected ion monitoring mode recording fragments at $m/\nu$ 245 and 246. 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 reciprocal pool model as described by Matthews *et al.* (17-19) for leucine kinetics. When this isotopic model is applied to $^{13}$C-valine, enrichment of plasma KIVA was assumed to provide an estimate of intracellular enrichment of valine (20). R(a) in µmol valine kg$^{-1}$ h$^{-1}$ was calculated according to the following equation:

$$R(a) = \frac{(Zi(V)/Z(KIVA) - 1) * i(V)}{eq(1)}$$

Where Zi(V) is the tracer-to-tracee ratio of the valine in the infusate, Z(KIVA) is the tracer-to-tracee ratio of KIVA in plasma, and i(V) is the infusion rate of $[1-^{13}$C]valine in µmol kg$^{-1}$ h$^{-1}$.

**Valine oxidation**

The rate of oxidation of valine was calculated following the approach by Van Goudoever
et al. (21) and described in an earlier study (11). In short, whole body bicarbonate flux is estimated using a short term primed continuous infusion of NaH$^{13}$CO$_3$ prior to infusion of $^{13}$C-valine. In this way, it is possible to obtain a two-point calibration in which one point is the background $^{13}$CO$_2$ enrichment at no infusion of NaH$^{13}$CO$_3$ and the second point is 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 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:

\[ i_{bic}(V) = i(b) \times \frac{Z_{co2}(V)}{Z_{co2}(B)} \]  

\[ \text{eq(2)} \]

In which $i_{bic}(V)$ is the $^{13}$C-bicarbonate production during valine infusion, $Z_{co2}(B)$ is the tracer-to-tracee ratio of expired air at isotopic steady state during the NaH$^{13}$CO$_3$ infusion, $Z_{co2}(V)$ is the tracer to-tracee ratio of expired air at isotopic steady state during the $^{13}$C-valine infusion, and $i(b)$ is the NaH$^{13}$CO$_3$ infusion rate in μmol kg$^{-1}$ h$^{-1}$. Using the KIVA tracer-to-tracee ratio in plasma, we calculated the amount of valine that needed to be oxidised to sustain this production of $^{13}$C-bicarbonate as follows:

\[ \text{Ox} = i_{bic}(V) \times 5 \times \left(\frac{100}{Z(KIVA)}\right) \text{ in } \mu\text{mol kg}^{-1} \text{ h}^{-1} \]  

\[ \text{eq(3)} \]

Where 5 is the number of carbon atoms in valine. This means that one can calculate the oxidation rate of an amino acid without measuring Vco$_2$.

During fasting:

\[ \text{Ox} = \text{O(fast)} \]  

\[ \text{eq(4)} \]

During feeding an increase in recovery of labelled CO$_2$ has been observed in comparison to fasting from 0.74 ± 7 to 0.84 ± 8, thus by approximately 13 % (22;23). This value has only been validated in control subjects. We adopted it for both the control subjects and the HD patients. Correction of the rate of oxidation of valine during feeding for this increased recovery is necessary since we obtained the two-point calibration by measuring $^{13}$CO$_2$ enrichment while the subject was fasting. O(fed) was calculated according to:

\[ \text{O(fed)} = \text{Ox} / 1.13 \]  

\[ \text{eq(5)} \]

**Calculation of whole body valine kinetics**

In figure 2, the isotopic model is depicted in a schematic diagram. The total rate of
The appearance of valine R(a) comprises the appearance of valine released from whole body protein breakdown (B), infusion of valine (i), and when appropriate dietary protein (I). The total rate of disappearance of valine comprises whole body protein synthesis (S) and whole body protein oxidation (O). The input fluxes in this model result in label dilution of infused [1-$^{13}$C]-valine in plasma. During fasting at steady state, the rate of appearance of valine equals the rate of disappearance of valine R(d)(24). During protein intake however, plasma amino acid concentrations increased gradually and therefore represent a non-steady state. The appearance of dietary valine into the circulation can then be considered to comprise a flux resulting in enlargement of the plasma valine pool ($\Delta Q$), and a flux of dietary valine (I) adding to whole body protein turnover (25). The flux of dietary valine (I) was calculated according to Wolfe (25). Valine intake (see Materials) was multiplied by 0.8 to correct for first pass metabolism both in control subjects and HD patients (26;27). The amount of dietary valine entrapped in the enlarged pool size of valine ($\Delta Q$) was calculated by multiplying the increase in plasma valine concentration by total body water (TBW). Total body water was calculated separately for the control subjects and HD patients. Hume-Weyer's equation (28) was used to estimate total body water in control subjects while Chertow's equation was used to estimate total body water in HD patients (29). The difference of plasma valine concentration before and at the end of the meal period was used to calculate the increase in concentration in the whole body valine pool. We observed that the increase of valine during dietary protein intake was continuous during our study.

**Figure 2.** 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), infusion of $^{13}$C-valine (i), and the dietary intake of valine (I).
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, corrected for the enlarged pool size of valine ($\Delta Q$).

At isotopic steady state:

$$R(a) = B + I + i = S + O = R(d),$$

all expressed in $\mu$mol valine kg$^{-1}$ h$^{-1}$. \textit{eq}(6)

During fasting, $I = 0$ so

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

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

During meal intake, $I \neq 0$ so

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

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

Protein balance was calculated by subtracting protein breakdown from protein synthesis.

\textit{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 kinetics due to the protein meal, fasting and fed state were compared using a two-tailed paired student t-test. Differences between HD patients and control subjects were tested using the two-tailed unpaired student t-test. Statistical significance was assumed when $p<0.05$.

\textit{Results}

\textit{Demographic and clinical data}

Table 1 shows the demographic and clinical data of the subjects studied. All patients were well nourished as can be concluded from the serum albumin concentrations while protein intake was appropriate. Dialysis was adequate as shown by the Kt/V values. There were no statistical differences between the two studied groups with respect to body mass index (BMI) or age. Albumin concentration was significantly higher in control subjects. During the 3 weeks preceding the day of the study, habitual protein and energy intake in controls and HD patients were not statistically different. Also, C-reactive protein (CRP) levels were not elevated (<2 mg l$^{-1}$) and plasma bicarbonate at the beginning of the previous hemodialysis session was larger than 21 mEq/l (mmol/l) which excludes
Table 1. Demographic, nutritional, and dialysis status of the studied HD patients and control subjects.

<table>
<thead>
<tr>
<th>Subjects</th>
<th>Control</th>
<th>Patient</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>45 ± 12</td>
<td>54 ± 10</td>
</tr>
<tr>
<td>Sex (male/female)</td>
<td>5/1</td>
<td>6/3</td>
</tr>
<tr>
<td>BMI (wt/m²)</td>
<td>23.6 ± 3.3</td>
<td>23.9 ± 3.4</td>
</tr>
<tr>
<td>Albumin (g/dl)</td>
<td>4.4 ± 0.1</td>
<td>4.1 ± 0.3*</td>
</tr>
<tr>
<td>Kt/V</td>
<td></td>
<td>1.2 ± 0.2</td>
</tr>
<tr>
<td>P.I. (g/kg/d)</td>
<td>1.0 ± 0.3</td>
<td>1.0 ± 0.2</td>
</tr>
<tr>
<td>E.I. (KJ/kg/d)</td>
<td>122 ± 34</td>
<td>111 ± 29</td>
</tr>
<tr>
<td>TBW (%)</td>
<td>57 ± 3</td>
<td>59 ± 5</td>
</tr>
</tbody>
</table>

1Mean ± SD. BMI: body mass index; to convert serum albumin g/dl to g/l, multiply by 10. Kt/V: Measure of dialysis adequacy; P.I.: Protein intake measured by dietary recording; E.I.: Energy intake measured by dietary recording. TBW: total body water as percentage of body weight. * Significantly different from control subjects, p<0.05.

Figure 3. Concentrations of total amino acids (TAA), essential amino acids (EAA) and non-essential amino acids (NEAA) in plasma during the steady state periods. Figure 3A represents the fasting state while figure 3B represents the fed state. * denotes a significant difference in amino acid concentration between control subjects and HD patients.
relevant metabolic acidosis in the patient population (30). Total body water was 57 ± 3 % for control subjects and 59 ± 5 % for HD patients (NS).

Primary data on concentrations and isotopic enrichment
In figure 3 the arterial plasma AA concentrations are shown for both study groups. In figure 3A, total AA, essential AA, and non-essential AA are shown for fasting patients and control subjects. There were no significant differences between the two groups. Figure 3B represents the meal period. There was a significantly higher essential AA concentration in the control group compared to the patient group. Furthermore, all amino acid concentrations in plasma were significantly higher during feeding compared to fasting for both groups.

The ratio of the isotopic enrichment of KIVA versus valine in plasma during fasting was 0.79 ± 0.04 and increased to 0.85 ± 0.08 during food intake in control subjects (p<0.05). In patients, the ratio was 0.77 ± 0.04 and increased to 0.84 ± 0.03 (p<0.05). There were no significant differences between control subjects and patients. In figure 4,
Table 2. Values of valine fluxes obtained by evaluation of primary data.

<table>
<thead>
<tr>
<th>Subjects</th>
<th>Fluxes (µmol val kg(^{-1}) h(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>R(a) Fasting</td>
</tr>
<tr>
<td>Control</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>99</td>
</tr>
<tr>
<td>2</td>
<td>88</td>
</tr>
<tr>
<td>3</td>
<td>80</td>
</tr>
<tr>
<td>4</td>
<td>91</td>
</tr>
<tr>
<td>5</td>
<td>85</td>
</tr>
<tr>
<td>6</td>
<td>91</td>
</tr>
<tr>
<td>Mean</td>
<td>89</td>
</tr>
<tr>
<td>SD</td>
<td>6</td>
</tr>
<tr>
<td>Patient</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>81</td>
</tr>
<tr>
<td>2</td>
<td>88</td>
</tr>
<tr>
<td>3</td>
<td>74</td>
</tr>
<tr>
<td>4</td>
<td>85</td>
</tr>
<tr>
<td>5</td>
<td>69</td>
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<tr>
<td>6</td>
<td>81</td>
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<tr>
<td>7</td>
<td>68</td>
</tr>
<tr>
<td>8</td>
<td>81</td>
</tr>
<tr>
<td>9</td>
<td>68</td>
</tr>
<tr>
<td>Mean</td>
<td>77</td>
</tr>
<tr>
<td>SD</td>
<td>8</td>
</tr>
</tbody>
</table>

R(a) is the total rate of appearance of valine, O(fast) and O(fed) are the oxidation rates during fasting and dietary protein intake, Intake is the estimated dietary valine intake corrected for first pass effect (see methods), \(\Delta Q\) is the entrapment of valine in the plasma amino acid pool.

Plateau enrichments for breath CO\(_2\) and plasma KIVA are shown to illustrate their steady state in time. Figure 4A shows, for the control group, the background enrichment (at \(t = 0\)), the plateau enrichment during the NaH\(^{13}\)CO\(_3\) infusion from 0.5 to 1 hour and the plateau enrichments during the \(^{13}\)C valine infusion while fasted (between 4 and 5 h) or fed.
Protein metabolism in hemodialysis patients

Figure 4B shows these same values for the patient group. Figure 4C shows, for the control group, the plasma KIVA plateau enrichment during fasting (between 4 and 5 h) and during the meal (between 7 and 8 h). Figure 4D represents these same values for the patient group.

Protein breakdown, synthesis, oxidation, and balance

In table 2, data on valine fluxes obtained by evaluation of primary data are given in control subjects and patients during both fasting and feeding. It can be seen that meal intake resulted in comparable dietary valine fluxes in patients and control subjects (93 ± 14 versus 89 ± 20 μmol kg⁻¹ h⁻¹) as well as comparable increases in plasma valine pool in both groups (37 ± 7 versus 30 ± 6 μmol kg⁻¹ h⁻¹). It is clear from this table however that the total rate of appearance of valine in plasma was significantly lower in HD patients compared to control subjects (fasting: 77 ± 8 versus 89 ± 6, p<0.01, meal intake: 108 ± 13 versus 123 ± 15 μmol kg⁻¹ h⁻¹, p<0.01). Therefore, as shown in table 3, protein breakdown was significantly lower in HD patients compared to control subjects both during fasting and feeding. Protein synthesis was also lower in HD patients compared to control subjects but this did not reach significance. Protein oxidation was reduced in HD patients compared to control subjects during the fasting period. The absolute and relative change compared to fasting in protein breakdown, synthesis and oxidation are also shown in table 3. There

### Table 3. Protein breakdown, synthesis, and oxidation in both control subjects and patients and the change in these parameters due to the meal.

<table>
<thead>
<tr>
<th>Whole body protein flux</th>
<th>Fasting</th>
<th>Meal intake</th>
<th>Change</th>
<th>Change (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Breakdown</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>81 ± 7</td>
<td>57 ± 11†</td>
<td>-24 ± 12#</td>
<td>-30 ± 14#</td>
</tr>
<tr>
<td>Patient</td>
<td>70 ± 8*</td>
<td>44 ± 10†*</td>
<td>-26 ± 12#</td>
<td>-36 ± 16#</td>
</tr>
<tr>
<td><strong>Synthesis</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>68 ± 8</td>
<td>89 ± 12†</td>
<td>21 ± 6#</td>
<td>30 ± 8#</td>
</tr>
<tr>
<td>Patient</td>
<td>62 ± 8</td>
<td>77 ± 11†</td>
<td>15 ± 7#</td>
<td>23 ± 11#</td>
</tr>
<tr>
<td><strong>Oxidation</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>21 ± 2</td>
<td>34 ± 7†</td>
<td>13 ± 6#</td>
<td>63 ± 30#</td>
</tr>
<tr>
<td>Patient</td>
<td>15 ± 5*</td>
<td>31 ± 5†</td>
<td>16 ± 7#</td>
<td>129 ± 86#</td>
</tr>
</tbody>
</table>

Last column is percentage change. All other values expressed as mean ± SD in μmol val kg⁻¹ h⁻¹. *p < 0.05 compared to control subjects. † p < 0.05 between fasting and meal intake. # p < 0.05 compared to 0 change.
were no significant differences of the mean values of these fluxes between HD patients and control subjects. Protein balance, calculated as the difference between the rates of whole body protein synthesis and breakdown, is shown in figure 5. Protein balance was significantly less negative in dialysis patients (-7 ± 5 μmol kg⁻¹ h⁻¹) compared to control subjects (-13 ± 2 μmol kg⁻¹ h⁻¹) during fasting. During feeding, protein balance was not different between patients (32 ± 9 μmol kg⁻¹ h⁻¹) and control subjects (31 ± 13 μmol kg⁻¹ h⁻¹). In HD patients, protein balance increased 39 ± 9 μmol kg⁻¹ h⁻¹ while this was 44 ± 12 μmol kg⁻¹ h⁻¹ in control subjects.

**Discussion**

The aim of the present study was to test the hypothesis that protein breakdown, synthesis, oxidation, and/or the anabolic response to a protein-enriched meal were perturbed in HD patients compared to control subjects. All studied subjects had been consuming a comparable protein intake for three weeks before the study protocol. Our data refuted our hypothesis and showed that ingested protein is handled similarly in HD patients and control subjects and that protein intake induced an identical anabolic response. However,
protein breakdown and oxidation during fasting and protein breakdown during meal intake were considerably depressed in HD patients compared to control subjects, suggesting lower metabolic activity in this patient group. These data suggest that protein utilisation is not impaired in stable HD patients on a non-dialysis day and that HD patients conserve their protein stores.

Before interpreting our results, we would like to discuss some methodological issues first. The use of amino acids, labelled with stable isotopes of N, C, or H to study whole body kinetics has been critically reviewed elsewhere (31;32). As discussed by Bier (31), the plasma valine turnover rate depends on the valine content of muscle protein similarly as has been found for leucine. In most studies [1-13C]-leucine has been used as a tracer while we used [1-13C]-labelled valine. Just like leucine, valine is an essential amino acid, which is metabolised very similar to leucine. Unlike leucine, valine infusion does not show an anabolic response by itself or in combination with insulin (33;34). Also, intravenous infusions of leucine depressed plasma levels of valine while intravenous infusions of valine did not affect leucine levels (35;36). Thus, although less often used, valine is as good a marker of whole body protein kinetics as leucine. A second issue is that calculation of true rates of whole body protein kinetics requires some sort of assessment of the isotopic enrichment of the intracellular precursor pool. Most commonly used in this respect is the reciprocal pool model by Schwenk et al. (37). In this model an intracellularly generated metabolite of the infused-labelled amino acid, which can be measured in plasma, is used as a marker for the intracellular enrichment of the infused-labelled amino acid. Since we used [1-13C]-labelled valine, isotopic enrichment of plasma KIVA, the transaminase product of valine was used to estimate intracellular enrichment of valine. The observed ratios of 13C-KIVA/13C-valine compared very favourable to those reported for the ratio of 13C-ketoisocaprate (transaminase product of leucine) over 13C-leucine (31) and to that reported by Sain-van der Velden et al. (38). The 13C-KIVA/13C-valine changed upon ingestion of a protein-enriched meal similarly as has been observed for the KICA/leucine ratio (31).

We used an independent infusion of NaH13CO3 of short duration to estimate whole body bicarbonate production in this study instead of indirect calorimetry with measurement of net CO2 production. Both methods have been compared previously and were shown to give similar values for net CO2 production (39). Still, we applied the 13C-bicarbonate method since the present study is part of a project in which whole body protein kinetics in HD patient was studied under a variety of conditions. Very recently a paper was published by our group in which the results were presented of a study which measured the anabolic
response on a protein meal in HD patients while dialysing (11). During dialysis, plasma bicarbonate exchanges with extra-corporeal bicarbonate in the dialysate. With indirect calorimetry, this effect of dialysis on the dynamics of whole body bicarbonate content can not be assessed appropriately, since this method measures only the net resulting effects of the change in dynamics.

We chose to study stable HD patients, who were well nourished and without co-morbidity, on a non-dialysis day. In this way we eliminated confounding factors due to malnourishment at the time of the study and co-morbidity. Since habitual protein intake greatly influences the magnitude of protein turnover (40), dietary protein intake in control subjects was reduced to 1.0 g kg\(^{-1}\) day\(^{-1}\) for three weeks to match the habitual intake of the HD patients. Habitual protein intake in the group of healthy control subjects was approximately 2 g kg\(^{-1}\) day\(^{-1}\) beforehand. A period of 3 weeks was applied because such a period of time is deemed necessary to allow for full adaptation of the body to a new level of protein intake (9). Patients and control subjects consumed self-selected diets with only a restricted protein intake. Energy intake was similar between patients and controls and intakes were normal for the age group albeit on the lower end (41). In this way, possible differences in whole body protein kinetics between HD patients and healthy control subjects can be assigned unequivocally to the renal insufficiency or long-term effects of its treatment.

Some concern might be raised whether absorption of the meal is the same in HD patients and in control subjects. In our patients, episodes of nausea, vomiting, abdominal distension and early satiety were absent or sporadic in the 3 months before the study period. This implies that gastric emptying in our patients is clinically normal (13). Also, the increase in amino acid concentrations in both control subjects and HD patients were similar and the enrichment of the KIVA pool was constant implying that in both groups protein breakdown was constant. This suggests that similar amounts of amino acids reached the circulation in our HD patients compared to healthy control subjects.

Returning to our results, we found that stable chronic HD patients were in a less catabolic state when measured during fasting on a non-dialysis day compared to control subjects. More specifically, both whole body protein breakdown and oxidation were significantly reduced in HD patients when compared to control subjects. In control subjects, it has been observed that whole body protein breakdown and oxidation are both dependent on activity levels (42;43). We did not assess activity levels in our studied subjects but it could well be the case that our HD patients were less active than our control subjects, thus possibly explaining the lower breakdown and oxidation. Similar observations of lower protein
breakdown and oxidation were made by Lim et al. (44) with L[1-13C]-leucine in nephrotic subjects, but their control subjects had a higher habitual protein intake compared to their patient group. Since the rate of protein turnover is dependent on habitual protein intake (9), the comparison between their patients and control subjects is more complicated. Also using 13C-leucine methodology, Berkelhammer et al. (7) however, found that in HD patients’ protein breakdown was unaffected, oxidation was increased and, accordingly, synthesis was reduced in these patients compared to control subjects. Since they did not report the plasma concentrations of leucine or any other amino acid or habitual protein intake, no unequivocal conclusions can be drawn from these observations.

We observed that the response to the ingested protein-enriched meal was not different between HD patients and healthy control subjects. Protein breakdown was substantially reduced while synthesis and oxidation were increased in both patients and control subjects. The reduction of protein breakdown accounts for the major part of the protein conservation both in HD patients and control subjects, while oxidation and synthesis are smaller contributors. Accordingly, protein balance is mainly regulated by changes in protein breakdown (10). Expressed as percentage change, there appears to be a more pronounced increase in oxidation in the HD patients compared to the control subjects, which can be explained by the depressed oxidation in the HD patients during the fasting period. An alternative explanation is that dialysis patients need greater caloric ingestion for protein conservation than controls.

Insulin as well as amino acid concentrations in plasma play important roles in the regulation of whole body protein metabolism (45;46). Both depress protein breakdown, but particularly increased amino acid concentrations stimulate protein synthesis and oxidation by increasing substrate availability. Insulin levels increased significantly and similarly in our patients and control subjects upon ingestion of the protein-enriched meal (data not shown). We also observed an almost twofold increase in plasma amino acid concentration. A difference with earlier studies is that we corrected for the enlarged valine pool. During the consumption of the meal, 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. This correction makes the calculation of whole body protein breakdown sensitive to changes in the plasma valine pool. Whole body protein synthesis is not influenced by this factor. We also assumed that enteral release was the same as the amount of ingested valine corrected by 20 % for first pass metabolism (26;27). This value was adopted for both the control subjects as the HD patients. However, the use of this value has not yet been validated in HD patients. Using the same value thus represents an oversimplification.
Protein-energy malnutrition might also be induced by the dialysis procedure itself. This was studied in an earlier paper by our group (11). We observed a more negative protein balance during dialysis. Ikizler et al (47) also observed that the dialysis procedure stimulates muscle and whole body protein loss. These adverse effects of dialysis on whole body protein balance could be reversed by the ingestion of a protein meal (11) or by intradialytic parenteral nutrition (48). The conclusion of these papers is that nutrition during dialysis is important. Long-term consequences have not yet been studied.

As a clinical consequence, our findings show that dialysis patients are capable of handling a substantial load of ingested protein well during a non-dialysis day. They are also able to incorporate these amino acids in body protein at a rate similar to control subjects. We did not investigate whether dialysis patients adapt as well as control subjects to a low protein intake. Tom et al. (49) proved that chronic renal failure patients adapt to a long-term low protein diet efficiently by reducing protein oxidation and by a postprandial inhibition of protein breakdown. Therefore it appears wise to provide adequate protein and energy intake to dialysis patients particularly during episodes of illness or surgery when food intake is low and protein breakdown is upregulated due to inflammatory conditions (50). Rather than a metabolic defect, relatively frequent hospital admissions caused by dialysis complications or co-morbidity may be the major cause of malnutrition in long-term hemodialysis patients.

In summary, we have shown that the rate of whole body protein breakdown is reduced in HD patients during a non-dialysis day both during fasting and feeding. Dialysis patients handle ingested protein just as efficiently as normal individuals. With sufficient dietary intake, it should be possible to keep stable hemodialysis patients in a neutral or even positive metabolic state. The notion that stable hemodialysis patients are in a protein catabolic state is thus not supported by this work.

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