Whole body protein metabolism in chronic hemodialysis

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Document Version
Publisher's PDF, also known as Version of record

Publication date: 2004

Link to publication in University of Groningen/UMCG research database

Citation for published version (APA):

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Chapter 2
Comparison of amino acid oxidation and urea metabolism in hemodialysis patients during fasting and meal intake

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Nephrology, Dialysis, and Transplantation, provisionally accepted
Abstract

Background. The PNA (protein equivalent of nitrogen appearance) is used to calculate protein intake from urea kinetics. One of the essential assumptions in the calculation of PNA is that urea accumulation in hemodialysis (HD) patients is equivalent to amino acid oxidation. However, urea is hydrolysed in the intestine and the resulting ammonia could be used metabolically. The magnitude and dependence on protein intake of this process are unknown in HD patients.

Methods. Seven HD patients were studied twice, one week apart on a similar protocol. After an overnight fast, patients fasted in the morning and received meals in the afternoon. On one day, amino acid oxidation was measured by infusion of L-[1-13C] valine. Urea production, measured from the dilution of 13C-urea, and urea accumulation, calculated from the increase in plasma urea concentration multiplied by the urea dilution volume were measured during the other day. PNA was calculated using standard equations.

Results. Amino acid oxidation and urea production were not significantly different during fasting. Urea accumulation during fasting was significantly lower than both amino acid oxidation and urea production. Urea accumulation during feeding remained significantly lower than amino acid oxidation. PNA was equal to the average of the urea accumulation values during fasting and feeding.

Conclusion. We conclude that during fasting, urea accumulation is not associated with amino acid oxidation or urea production. During meal intake, amino acid oxidation, urea production, and urea accumulation show acutely an almost identical increase. PNA represents the average of fasting and fed urea accumulation and is lower than average amino acid oxidation or urea production.

Keywords: Stable isotope, urea dilution volume, urea kinetics, PNA.
Figure 1. Whole body nitrogen metabolism is schematically represented showing the interactions of amino acid oxidation, urea production, and urea accumulation in hemodialysis patients. Solid lines represent measured values from the results; dotted lines represent possibilities mentioned in the discussion.

Introduction

It is well known that the clinically used protein equivalent of nitrogen appearance (PNA) represents only that amount of protein that is irreversibly oxidised. It is measured as the difference in urea concentration in blood of HD patients between 2 dialysis sessions. The older term “protein catabolic rate” has been discarded, since a substantial portion of metabolised protein is simply broken down into amino acids, which are subsequently used to synthesise new proteins. A question is whether nitrogen appearance i.e. the urea appearing in the urine or in the rising urea concentration of hemodialysis patients between their hemodialysis sessions is only influenced by amino acid oxidation. Urea production is the main route by which the body discards excess nitrogen. Not all urea is excreted as such and some nitrogen of urea can be released again as ammonia through the metabolic activity of the colonic microflora (1;2). Estimates of this process range from 14 to 60 % of the urea production depending on experimental conditions (3-5). This newly formed ammonia could then be reutilized in amino acid synthesis and incorporated into newly formed proteins either by intestinal bacteria (6) or by the liver (7) as has been observed in healthy humans. These observations indicate that the supposedly linear sequence of
Intestinal metabolism of urea leads to intestinal ammonia formation which re-enters whole body ammonia metabolism at various stages and to a various extent, depending on the nutritional status of the studied subjects. It is known that this process takes place under diverse conditions both in healthy subjects and chronic renal failure patients (8;9) but the extent is not well defined. In renal failure patients, studies measuring nitrogen balance during fasting did not include data on the anabolic response to a meal. In normal men, two groups looked at $^{13}$C-leucine oxidation and urea metabolism both during fasting and feeding (10;11). Urea metabolism was measured using nitrogen balance studies in one study (11) while in the other, $^{15}$N$^{15}$N-urea was used to estimate nitrogen flux (10). The authors concluded that urea hydrolysis and ammonia recycling occurred especially during fasting but that there was no salvage of nitrogen from the colonic microflora in normal man during a feeding period. Thus depending on the source of protein, the relative contribution of the different pathways resulting in urea accumulation, as depicted in figure 1, can vary considerably. The question arises how urea accumulation, urea production and amino acid oxidation are related during fasting when body proteins are being oxidised and during protein intake when nutritional proteins are oxidised. We conducted the present studies in order to: determine the extent of amino acid oxidation, urea production, and urea accumulation during a fasting and a feeding period separately and 2: evaluate the relationship between PNA, urea kinetics (12), and amino acid oxidation. Amino acid oxidation was estimated from measurements of $^{13}$CO$_2$ appearance in expired air using $^{13}$C-labeled valine. Urea production and the urea dilution volume (UDV) were measured using $^{13}$C-urea dilution (13). Urea accumulation was calculated from the measured increase of urea concentration in plasma during fasting and feeding separately multiplied by the UDV measured from the $^{13}$C-urea dilution method.

**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 this protocol. Seven patients, 2 females and 5 males, gave their permission. The medical ethical committee of the University Hospital of Groningen approved all studies and written informed consent was obtained from all participants. Two patients had been diagnosed as having chronic glomerulonephritis (one...
with hypertension), three had nephropathy due to hypertension, one had polycystic kidney disease and one quiescent Wegeners disease. All patients had been clinically stable for more than 3 months before the study protocol. Medications included phosphate binders, iron, multivitamins, anti-hypertensive drugs, calcitriol, and in six patients recombinant human erythropoietin. No patients received hormone or immunosuppressive agents for 6 months before the study days. 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\(^{-1}\). All patients used standard dialysate with 140 mEq Na\(^+\), and 34 mEq bicarbonate. Residual renal function was 6 ml min\(^{-1}\) in 1 patient and less than 3 ml min\(^{-1}\) in the other 6 patients and urinary flow was 500 ml per 24 hours for 1 patient, below 400 ml per 24 hours for 2 patient, and none in 4 patients. 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\(^{-1}\). During the study, blood pressure and hydration status were monitored.

**Materials**

L\([1^{-13}\text{C}]\) valine, NaH\([^{13}\text{C}]\)O\(_3\), and \([^{13}\text{C}]\)-urea (>99 % atom percent enrichment respectively) were purchased from Cambridge Isotope Laboratories, (Andover, Ma, USA). Chemical purities were confirmed before use. Pyrogen and bacteria free solutions were prepared in sterile saline by the hospital dispensary. Meal portions consisted of 150 grams of yoghurt (5.7 g protein, 7.4 g carbohydrate, and 5.4 g fat, Domo, The Netherlands), 20 grams of cream (0.5 g protein, 0.7 g carbohydrate, and 6.3 g fat Friesche vlag, Ede, the Netherlands) and 5 grams of protein enriched milk powder (1.5 g protein, 2.4 g carbohydrate, and 0.8 g fat, Fortify, Nutricia, The Netherlands). Consumption of a meal portion every 30 minutes for 3 hours resulted in a dietary valine intake of 9.8 mmol h\(^{-1}\) 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 the dialysis procedure since our patients had no history of dyspeptic symptoms during the three months before both protocols.

**Experimental design**

In the amino acid oxidation protocol, patients were fasted overnight and were studied during a midweek day without dialysis, having dialysed the afternoon before. Patients were admitted to the Hospital Research Unit at ≈ 7:30 AM. A catheter was inserted into the dorsal vein of the hand of the shunt arm to collect baseline blood samples. Breath samples were taken simultaneously into 10 ml glass containers. A schematic diagram of
Figure 2. Schematic representations of both study days. 2A protocol used to study whole body protein metabolism in chronic hemodialysis patients during a non-dialysis day. After an overnight fast, whole body amino acid oxidation was measured in the morning while fasting. This was followed in the afternoon by the measurement of whole body amino acid oxidation in patients consuming a protein- and energy enriched meal in 6 portions. 2B protocol used to study whole body urea production and urea accumulation in chronic hemodialysis patients. A bolus $^{13}$C-urea was given at 0800 hour in the morning and steady state was reached within 120 minutes. After the overnight fast, urea production and urea accumulation were measured in the morning during fasting. Fed urea production and accumulation were measured in the afternoon during intake of 6 small meals.

The metabolic study day is shown in figure 2A. The NaH$^{13}$CO$_3$ infusion was started at 8:00 AM. During the first hour whole body bicarbonate flux was measured using a primed constant infusion of $^{13}$C labelled bicarbonate (5 µmol kg$^{-1}$ bolus followed by a continuous infusion of 5 µmol kg$^{-1}$ h$^{-1}$). Four breath samples were taken between 30 and 60 min at 10 min intervals 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. A second catheter was now inserted in the contralateral arm to collect blood samples. Blood and breath samples were taken simultaneously every half hour for two 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, the meal was started by consumption of the first portion of the protein- and energy enriched meal and continued for 3 hours by consumption of a portion every 30 minutes. The infusion of $^{13}$C-valine was continued 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 were observed until stable and then discharged after approximately 30 minutes. After one week,
the same 7 subjects were studied according to a 7-hour tracer protocol, partly similar to the first day (figure 2B). Patients received a single bolus injection of 10 µmol $^{13}$C-urea kg$^{-1}$ in approximately one-minute at 08:00 AM. Blood samples were taken during the whole study day for the measurement of urea concentrations and urea enrichment at the same time points described above. Meal intake started at 12:00 AM and continued for three hours. The study day ended at 3:00 PM, all catheters were removed and patients were observed until stable and then discharged after approximately 30 minutes. The two study periods were randomised.

**Analytical methods**

Four ml of blood was drawn for each sample in liquid heparinized vacuum tubes and centrifuged at 3000 RPM. Plasma was extracted and stored at -20º 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. Urine samples, when produced, were collected during the study day and their volume and urea concentrations (micro Kjeldahl analysis) were measured to calculate total excreted urea. These values were then added to the urea accumulation numbers. Albumin concentrations were determined by standard clinical chemistry methods. Determination of valine oxidation requires $^{13}$C enrichment measurements for breath CO$_2$, plasma valine and plasma keto-isovaleric acid (KIVA), an intra-cellular metabolite of valine. Amino acids were isolated from plasma using a cation exchange column (SCX-100, 209800, Alltech, Deerfield, Il, USA) and converted to the N(O)-methoxycarbonyl methyl ester (MCF) derivative. The determination of $[1-^{13}$C] KIVA isotopic enrichment was done as described earlier (14).

**Calculations**

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.* (15) for leucine kinetics. In our isotopic model, enrichment of plasma KIVA is assumed to provide an estimate of intracellular enrichment of valine as described earlier (14). R(a) in µmol valine kg$^{-1}$ h$^{-1}$ was calculated according to the following equation:

$$
R(a) = \frac{MPE_i(V)}{MPE(KIVA)} - 1 \times i(V)
$$

Where MPE$_i$(V) is the isotopic enrichment of the valine in the infusate in mole percent excess, MPE(KIVA) is the isotopic enrichment of KIVA in plasma in mol percent excess,
and i(V) is the infusion rate of $[1^{-13}C]$valine in $\mu$mol kg$^{-1}$ h$^{-1}$. The rate of oxidation of valine was calculated applying the approach described in detail earlier (14). 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. This flux was subsequently used to estimate the $^{13}$C-bicarbonate flux originating from the oxidation of $^{13}$C-valine. Amino acid oxidation values were converted to urea production values. For this purpose, we assumed that valine content of protein is 5.5 g per 100 g of muscle protein. This 5.5 g corresponds to 47 mmol valine. We also assumed that 100 g of protein contains 16 g of nitrogen, which corresponds to 1.14 mole N. Oxidation of an amount of protein, containing 1 mole of valine produces $1140 / 47 = 24$ moles of nitrogen, which corresponds to 12 moles of urea assuming that initially, all nitrogen is converted to urea. The oxidation of 1 mole of valine produces 1 mole of NH$_3$ and rates of amino acid oxidation, calculated as $\mu$mol val kg$^{-1}$ h$^{-1}$ were converted into $\mu$mol urea equivalents kg$^{-1}$ h$^{-1}$ by multiplying with a factor 12.

Urea production was measured by isotope dilution of $[^{13}C]$ urea as described earlier (13). In short, urea enrichment of the plasma samples of each subject was calculated using the slope and intercept of the calibration lines prepared in plasma samples obtained before the bolus injection of $[^{13}C]$urea. The fasting state was defined as the third hour after infusion, while the fed state was defined as the second and third hour after the start of the meal ingestion. For the calculations, urea dilution volume, plasma urea concentration, and the synthetic rate of urea during fasting and during the last two hours of feeding were measured and a steady state during these periods was assumed. The calculations of UDV and the calculations of urea production were performed as described earlier (13). Nitrogen losses via the sweat were assumed to be negligible (16) as were faecal losses (17).

Amino acid concentrations in plasma were measured by the AccQ Tag method using HPLC according to the manufacturer’s protocols (Waters, Breda, The Netherlands) during the last hour of the fasting and the last hour of the feeding period.

Urea accumulation, measured during the non-dialysis study days, was calculated by the increase of the plasma urea concentration per hour, multiplied by the urea dilution volume calculated by standard equations (12;13) and expressed per kilogram bodyweight. Urea hydrolysis was defined as urea production minus urea accumulation.

PNA was determined from the rise in plasma urea concentration during the interdialytic interval and the measured urea distribution volume. Calculations were performed as described earlier (12). PNA was normalised to the actual post-dialysis dry weight of the patient.
**Statistics**

All values are given as means ± SD. To compare the influence of the protein meal, the fasted and fed states were compared using paired student t-test. Differences between methods were compared using the same test. Statistical significance was assumed when p<0.05. Correlations between the methods were tested using the Pearson’s correlation method in SPSS version 10 (SPSS inc. Chicago, Illinois, USA). The average slope was calculated from the individual slopes and was tested as a one-sample t-test versus the line of identity or a slope of 1. The average intercept was calculated from the individual intercepts and was tested as a one-sample t-test versus 0.

**Table 1: Demographic, nutritional, and dialysis status of the study patients.**

<table>
<thead>
<tr>
<th>Patients</th>
<th>Age/sex</th>
<th>BMI</th>
<th>Albumin</th>
<th>Kt/V</th>
<th>UDV</th>
<th>Urea</th>
<th>Renal Kt/V</th>
<th>P.I.</th>
<th>E.I.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>wt/m²</td>
<td>G/l</td>
<td>L</td>
<td>mmol/l</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>62/M</td>
<td>22.2</td>
<td>36</td>
<td>1.52</td>
<td>37.4</td>
<td>12.4</td>
<td>0</td>
<td>0.91</td>
<td>29.4</td>
</tr>
<tr>
<td>2</td>
<td>64/M</td>
<td>26.3</td>
<td>43</td>
<td>1.20</td>
<td>43.3</td>
<td>14.3</td>
<td>0</td>
<td>0.84</td>
<td>22.4</td>
</tr>
<tr>
<td>3</td>
<td>58/F</td>
<td>21.2</td>
<td>38</td>
<td>1.32</td>
<td>25.6</td>
<td>16.0</td>
<td>0</td>
<td>1.13</td>
<td>31.4</td>
</tr>
<tr>
<td>4</td>
<td>39/M</td>
<td>18.7</td>
<td>40</td>
<td>1.11</td>
<td>43.3</td>
<td>16.3</td>
<td>0.45</td>
<td>1.17</td>
<td>38.3</td>
</tr>
<tr>
<td>5</td>
<td>56/F</td>
<td>23.4</td>
<td>40</td>
<td>1.12</td>
<td>44.7</td>
<td>21.7</td>
<td>0.16</td>
<td>1.00</td>
<td>21.7</td>
</tr>
<tr>
<td>6</td>
<td>59/M</td>
<td>30.7</td>
<td>44</td>
<td>1.25</td>
<td>26.0</td>
<td>10.0</td>
<td>0</td>
<td>0.80</td>
<td>20.5</td>
</tr>
<tr>
<td>7</td>
<td>35/M</td>
<td>25.0</td>
<td>42</td>
<td>1.30</td>
<td>42.0</td>
<td>9.8</td>
<td>0.2</td>
<td>0.66</td>
<td>16.9</td>
</tr>
<tr>
<td>Average</td>
<td>53</td>
<td>23.9</td>
<td>40</td>
<td>1.2</td>
<td>38</td>
<td>14.4</td>
<td>0.11</td>
<td>0.93</td>
<td>25.8</td>
</tr>
</tbody>
</table>


**Results**

Table 1 shows demographic and clinical data of the patients studied. Body weight remained stable during the three weeks before the experiments. C-reactive protein concentration was less than 2 mg/l in all patients during the three months before the study. During the measurement of urea and valine kinetics, a stable metabolic state was achieved (data not shown). UDV was 50 ± 8 % of body weight, which was within the normal range. Valine concentrations during the urea infusion day were lower than during the valine infusion day both during fasting and meal intake, which can be explained by the
Urea kinetics and protein metabolism

Figure 3. (A) Plasma \([^{13}\text{C}]\)ketoisovaleric acid (KIVA) enrichment, steady state enrichments in KIVA are shown during fasting (5th hour) and meal intake (8th hour). (B) the percentage change in urea concentration in time is shown compared to the average of the first 30 minutes. The error bar at 30 minutes represents the interindividual differences while the other data points represent the intra-individual differences compared to the time point at 30 minutes. (C) the percentage change in urea enrichment is shown compared to the enrichment in plasma after 2 hours. The error bar at 2 hours represents the Inter-individual difference, which was due to the differences in urea pool size. The other data points represent the Intra-individual differences compared to the time point at 2 hours.

13C-valine infusion. Protein intake was 15 g protein/h, which accounted for an intake of 0.2 g protein kg\(^{-1}\) h\(^{-1}\). Conversion of this value to urea equivalents resulted in an intake of 1100 µmol urea N kg\(^{-1}\) h\(^{-1}\). Amino acid concentrations in plasma increased except for histidine, citrulline (significant decrease), glutamine, glutamic acid, glycine and taurine (table 2). This increase is equivalent to 54 mmol amino acid N per 3 hours, which is equal to 125 µmol urea N kg\(^{-1}\) h\(^{-1}\).

In figure 3A, steady state enrichments in KIVA are shown during fasting (5th hour) and meal intake (8th hour). In figure 3B, the percentile change in urea concentration in time is shown compared to the average of the first 30 minutes. The error bar at 30 minutes represents the interindividual differences while the rest of the data points represent the
Table 2. Amino acid concentrations during fasting, meal intake and the difference over the 3 hour period.

<table>
<thead>
<tr>
<th>Amino acid</th>
<th>[AA] during fasting</th>
<th>[AA] during meal intake</th>
<th>Change in [AA]</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Essential</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Histidine</td>
<td>72 ± 19</td>
<td>86 ± 23</td>
<td>14 ± 15</td>
</tr>
<tr>
<td>Iso-leucine</td>
<td>57 ± 17</td>
<td>127 ± 19</td>
<td>70 ± 12**</td>
</tr>
<tr>
<td>Leucine</td>
<td>110 ± 38</td>
<td>232 ± 34</td>
<td>123 ± 15**</td>
</tr>
<tr>
<td>Lysine</td>
<td>147 ± 34</td>
<td>249 ± 27</td>
<td>102 ± 49**</td>
</tr>
<tr>
<td>Phenyl-alanine</td>
<td>52 ± 16</td>
<td>99 ± 15</td>
<td>47 ± 7**</td>
</tr>
<tr>
<td>Threonine</td>
<td>103 ± 25</td>
<td>157 ± 36</td>
<td>54 ± 38**</td>
</tr>
<tr>
<td>Valine</td>
<td>223 ± 44</td>
<td>375 ± 41</td>
<td>152 ± 28**</td>
</tr>
<tr>
<td><strong>Nonessential</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alanine</td>
<td>325 ± 98</td>
<td>513 ± 145</td>
<td>189 ± 157**</td>
</tr>
<tr>
<td>Arginine</td>
<td>75 ± 16</td>
<td>99 ± 16</td>
<td>23 ± 11**</td>
</tr>
<tr>
<td>Asparagine</td>
<td>40 ± 9</td>
<td>67 ± 29</td>
<td>27 ± 26*</td>
</tr>
<tr>
<td>Aspartic acid</td>
<td>25 ± 8</td>
<td>31 ± 7</td>
<td>6 ± 6*</td>
</tr>
<tr>
<td>Cysteine</td>
<td>50 ± 19</td>
<td>58 ± 21</td>
<td>8 ± 4**</td>
</tr>
<tr>
<td>Citruline</td>
<td>120 ± 18</td>
<td>107 ± 24</td>
<td>-12 ± 13*</td>
</tr>
<tr>
<td>Glutamine</td>
<td>531 ± 116</td>
<td>539 ± 108</td>
<td>8 ± 115</td>
</tr>
<tr>
<td>Glutamic acid</td>
<td>54 ± 33</td>
<td>95 ± 76</td>
<td>41 ± 54</td>
</tr>
<tr>
<td>Glycine</td>
<td>360 ± 186</td>
<td>338 ± 189</td>
<td>-22 ± 43</td>
</tr>
<tr>
<td>Methionine</td>
<td>19 ± 5</td>
<td>40 ± 12</td>
<td>21 ± 12**</td>
</tr>
<tr>
<td>Ornithine</td>
<td>58 ± 14</td>
<td>76 ± 19</td>
<td>18 ± 15**</td>
</tr>
<tr>
<td>Proline</td>
<td>286 ± 116</td>
<td>562 ± 213</td>
<td>276 ± 115**</td>
</tr>
<tr>
<td>Serine</td>
<td>71 ± 22</td>
<td>102 ± 33</td>
<td>31 ± 27**</td>
</tr>
<tr>
<td>Taurine</td>
<td>56 ± 28</td>
<td>56 ± 20</td>
<td>1 ± 16</td>
</tr>
<tr>
<td>Tyrosine</td>
<td>36 ± 12</td>
<td>80 ± 16</td>
<td>45 ± 15**</td>
</tr>
<tr>
<td><strong>Total amino acids (mM)</strong></td>
<td>127 ± 18</td>
<td>181 ± 22</td>
<td>54 ± 24**</td>
</tr>
</tbody>
</table>

All values in µmol/l except total amino acids, which is in mmol/l. * p < 0.05 fasting compared to feeding. ** p < 0.01 fasting compared to feeding.
intra-individual differences compared to the time point at 30 minutes. During meal intake, these differences became somewhat larger due to the large inter-individual differences in urea accumulation. In figure 3C, the percentile change in urea enrichment is shown compared to the enrichment in plasma after 2 hours. The error bar at 2 hours represents the inter-individual difference, which was due to the differences in urea pool size. The rest of the data points represent the intra-individual differences compared to the time point at 2 hours. Only one patient produced a significant volume of urine. Patient 4 had a urine production of 350 ml during the whole study day during both the fasting and meal period with a urea concentration of 30 mmol l\(^{-1}\). This reflects a urea excretion rate of 1.75 mmol h\(^{-1}\) equivalent to 23 mmol urea N kg\(^{-1}\) h\(^{-1}\). The other six patients did not produce urine during the study day.

The individual results of PNA, protein oxidation, urea production, and urea accumulation in the 7 patients are presented in table 3 and the mean results in figures 4A and B. During
fasting (fig 4A), urea production and amino acid oxidation yielded similar results. Urea accumulation (plus excretion) was significantly lower than the other two values. During meal intake (fig 4B), urea production and amino acid oxidation were not significantly different but urea accumulation remained significantly lower than amino acid oxidation. The increase in all three parameters due to the meal is shown in figure 4C. PNA, calculated over the whole interdialytic interval, was $218 \pm 25 \mu\text{mol urea N kg}^{-1} \text{h}^{-1}$ (table 3), equivalent to $1.1 \pm 0.1 \text{g protein kg}^{-1} \text{day}^{-1}$. This was not significantly different from protein intake estimated from the dietary diary (table 1).

Figure 5 shows that there is a systematic difference between urea accumulation and the other two parameters. All parameters were significantly correlated (oxidation and urea accumulation $r = 0.802$, $p < 0.01$ Fig. 5A, production and accumulation $r = 0.771$, $p <$
0.01 Fig. 5B, production and oxidation $r = 0.538$, $p < 0.05$ Fig. 5C). The intercept with the Y-axis in figure 5A and B is significantly higher than 0 (represented in the formula in each graph) and for figure 5C was not different from 0. The slope (also represented in the formula in each graph) of all three correlations was not significantly different from 1.

Discussion

The main findings of this study were that during fasting, urea production and amino acid oxidation were higher than urea accumulation values implying urea hydrolysis in HD patients up to almost 50% (10). This indicates that especially during fasting urea hydrolysis is of quantitative importance (3;18). All parameters increased significantly after meal intake with greater dispersion of values. During meal intake, urea accumulation was significantly different from amino acid oxidation but not from urea production. Long-term PNA calculations were comparable to time-averaged urea accumulation values for fasting and feeding.
We combined valine oxidation values, estimates of urea production measured with $^{13}$C-urea and compared these with experimentally derived values of urea accumulation during a fasting and a feeding period. The oxidation rate of valine was used to calculate whole body amino acid oxidation, which we subsequently converted into values of urea metabolism. This procedure has been validated in healthy control subjects (5). Dilution of $^{13}$C-labeled urea is a sensitive method to measure total urea production. It has been used previously in our laboratory to calculate urea distribution volume and urea kinetics (13). By combining these values for valine oxidation and urea metabolism, we thought we might be able to specify the relative contributions of the various processes to whole body nitrogen flows, as shown in figure 1 in more detail. During fasting, when nitrogen intake was zero, amino acid oxidation was found to be equivalent to 200 µmol urea N kg$^{-1}$ h$^{-1}$. This is comparable to the value of 159 µmol urea N kg$^{-1}$ h$^{-1}$ (61 mg N/kg/12 hour) during fasting reported in the study by el Khoury et al. (10). Urea production in our study was 190 µmol urea N kg$^{-1}$ h$^{-1}$, which is not significantly different from the amino acid oxidation. Earlier studies showed a similar agreement between amino acid oxidation and urea production in control subjects (3;19). However, we observed that the rate of urea accumulation was only approximately 100 µmol urea N kg$^{-1}$ h$^{-1}$. This raises the question as to where this excess urea production of $\sim$100 µmol urea N kg$^{-1}$ h$^{-1}$ has gone. As has been shown in control subjects and pre-dialysis patients, up to 50% of produced urea can be hydrolysed in the intestines (3). Varcoe et al. (20) showed a significant recycling of urea, which was confirmed by Mitch (8) in pre-dialysis patients. Theoretically, if all ammonia arising during intestinal urea hydrolysis would result in intrahepatic urea production, this would imply a total urea production of 300 µmol urea N kg$^{-1}$ h$^{-1}$, comprising urea production due to amino acid oxidation and urea hydrolysis. Since we observed a value of approximately 200 µmol urea N kg$^{-1}$ h$^{-1}$ for the rate of urea production, alternative routes need to be assumed for deposition of intestinally arising ammonia (or detoxification). Ammonia, formed by intestinal hydrolysis of urea could be incorporated into the amino acid pool as was shown by others in control subjects (6). We suggest that intestinal ammonia is used in the production of (non-)essential amino acids, either by the colonic microflora, or by hepatic metabolism in chronic hemodialysis patients. On the other hand, one should realise that it might also indicate that amino-N, arising during amino acid oxidation is not directed exclusively into urea formation (2;7). With the current data, these two routes can not be studied separately.

During protein intake, amino acid concentrations increased in our patients and so did amino acid oxidation, urea production, and urea accumulation. The increase in amino acid concentration represented an N retention equivalent to 125 µmol urea N kg$^{-1}$h$^{-1}$, which
was equal to 11% of the total protein intake (1100 µmol urea N kg$^{-1}$h$^{-1}$). Amino acid oxidation during meal intake increased to 441 µmol kg$^{-1}$ h$^{-1}$. Thus half of the ingested protein is not immediately used for protein synthesis and 40% of this half is immediately oxidised. In the urea pool, most of these oxidised amino acids are found in urea production values \textit{i.e.} 360 µmol kg$^{-1}$ h$^{-1}$. Of these, 300 µmol kg$^{-1}$ h$^{-1}$ is retained in the urea pool in body water in the hemodialysis patient. Thus, after meal intake amino acid oxidation, urea production, and urea accumulation all increase approximately 200 µmol kg$^{-1}$ h$^{-1}$ (see figure 3C) and the differences between these values are no longer significant during meal intake. This is partly explained by the larger variability in all three parameters. Also, we suggest that intestinal bacteria could decrease their utilisation of urea as a fuel and start using food derived components. This decreases urea hydrolysis thus reducing the difference between urea accumulation and the other two parameters. This is confirmed by the offset in the correlation between amino acid oxidation/urea production and urea accumulation of approximately 150 µmol kg$^{-1}$ h$^{-1}$, which represents the amount of urea that can be hydrolysed in the intestinal microflora in stable hemodialysis patients and reused for other purposes.

We can conclude that in hemodialysis patients with little urinary urea excretion, there is agreement during fasting between amino acid oxidation and total urea production but these values do not agree with urea accumulation during fasting. Amino acid oxidation, urea production, and urea accumulation show an acute response to meal intake and the differences between the three parameters diminish with meal intake. PNA represents the time-averaged urea accumulation for fasting and meal intake but is lower compared to the average amino acid oxidation or urea production.
Acknowledgements

This work was supported by a grant from the Dutch Kidney Foundation (C 97-1694). The authors would like to thank Henk Elzinga for analysis of the breath samples. The authors also appreciate the time from the patients and staff of the Dialysis Centre Groningen and the outpatient renal function ward. Part of this work was presented at the 34th annual meeting of the American Society of Nephrology, 2001.
Chapter 2

Urea kinetics and protein metabolism

Reference list


