Protein metabolism in adult patients with Phenylketonuria

Margreet van Rijn R.D. a)
Marieke Hoeksma M.D. a)
Pieter Sauer M.D., Ph.D. a,c)
Beate Szczerbak Ph.D d)
Martina Gross M. Sc. d)
Dirk-Jan Reijngoud Ph.D. c,e)
Francjan van Spronsen M.D., Ph.D. a,c)

a) Section of Metabolic Diseases, Department of Pediatrics, Beatrix Children’s Hospital, University Medical Center Groningen, University of Groningen, Groningen, The Netherlands
b) Department of Pediatrics, Beatrix Children’s Hospital, University Medical Center Groningen, University of Groningen, Groningen, The Netherlands
c) Center for Liver, Digestive and Metabolic Diseases, University Medical Center Groningen, University of Groningen, Groningen, The Netherlands
d) Milupa GmbH, Friedrichsdorf, Germany (BS, MG).
e) Research Laboratory of Paediatrics, University Medical Center Groningen, University of Groningen, Groningen, The Netherlands

ABSTRACT

Background: Protein intake recommendations in Phenylketonuria (PKU) are frequently subject of discussion. For healthy adults, the Recommended Daily Allowance (RDA) is 0.8 g·kg\(^{-1}\)·d\(^{-1}\), which is generally lower than observed in the general western population.

Objective: To study whether whole body protein metabolism in PKU patients is comparable to healthy controls at a RDA rate of protein intake.

Design: Six adult well-controlled PKU patients and 6 healthy individuals of comparable age, height and weight were studied using a primed-continuous infusion of [1-\(^{13}\)C]-valine for 8h after an overnight fast before and during frequent meals. Normal protein was given to controls, whereas PKU patients received a combination of an amino acid mixture and natural protein.

Results: No significant differences were observed between PKU patients and controls in preprandial (pp) and prandial (p) rates of valine appearance and oxidation and protein breakdown (B), protein synthesis (S) and net protein balance (NPB). Feeding resulted in a significant (\(P<0.01\)) decrease of B (PKU: 94±15 (pp) to 49±10 (p); controls: 97±10 (pp) to 55±10 (p) \(\mu\text{mol}\cdot\text{kg}^{-1}\cdot\text{h}^{-1}\)), whereas no effects were observed in S (PKU: 77±10 (pp) to 73±7 (p); controls: 76±8 (pp) to 71±5 (p) \(\mu\text{mol}\cdot\text{kg}^{-1}\cdot\text{h}^{-1}\)). NPB increased from negative (p) to positive (pp) values (PKU: –17±6 (pp) to +23±8 (p); controls: –21±4 (pp) to +16±9 (p) \(\mu\text{mol}\cdot\text{kg}^{-1}\cdot\text{h}^{-1}\)).

Conclusion: Whole body protein metabolism in adult PKU patients is fully comparable to healthy controls at the RDA level of protein intake.

KEYWORDS: Phenylketonuria, protein requirement, amino acid oxidation, whole body protein turnover, stable isotopes, [1-\(^{13}\)C]-valine, L-[1-\(^{13}\)C] ketoisovaleric acid (KIVA).

INTRODUCTION

Patients with phenylketonuria (PKU, McKusick 261600) cannot convert phenylalanine (Phe) into tyrosine (Tyr) due to a deficiency of phenylalanine hydroxylase (EC1.14.16.1) activity in the liver. Left untreated, PKU leads to high Phe concentrations in blood and tissues and low to normal Tyr concentrations, clinically resulting in severe mental retardation, epilepsy and behavioral problems\(^1\). Treatment consists of restriction of the essential amino acid Phe by reducing the natural protein intake with concomitant supplementation of all amino acids but Phe. Patients, treated by this dietary Phe restriction have a more or less normal outcome although some minor neuropsychological dysfunction remains\(^1-3\). Recommendations about the optimal amount of supplementation of the amino acids are based on protein recommendations for healthy individuals and factors that may influence optimal protein intake in PKU. Studies have been carried out about nutritional value of free amino acids as compared to natural protein, about growth in young PKU patients and about metabolic control in PKU patients\(^4-18\). The question arose as to whether the results of all these studies are suitable to determine the amount of amino acid supplementation for adults. The number of adult PKU patients on diet is still growing since the first PKU patients have reached adulthood after the start of newborn screening and the issue of evidence for optimal protein recommendations requires attention. Lifelong dietary treatment with high level supplementation of
amino acids may be unnecessary and has several drawbacks both economically and socially, since amino acid supplements are expensive and adherence to the intake prescription is difficult. The aim of the present study was to compare whole body protein metabolism in healthy adults and adult PKU patients preprandial and a subsequent prandial period at a protein intake comparable to the RDA of 0.8 g · kg⁻¹ · day⁻¹. Our hypothesis was that protein metabolism in PKU patients is intrinsically not different from healthy controls.

SUBJECTS AND METHODS

Study subjects:
Six patients with PKU and 6 healthy adult individuals were studied. The 6 PKU patients (males and females) had a mean age of 27 ± 7 years with a normal height (mean Z score -0.6 ± 1), and a mean body weight of 70 ± 9 kg and a mean body mass index (BMI) of 23 ± 3 (kg/m²) (Table 1). All patients had an intellectual development within the normal range. Tolerance of dietary Phe (based on daily intake of natural protein) at 5 years of age was 21 ± 9 mg Phe · kg⁻¹ · d⁻¹ and 11 ± 4 mg Phe kg⁻¹ · day⁻¹ at the time of the test. Treatment of patients was considered adequate as the mean Phe concentration was 522 ± 106 μmol/L during the past 2 years and the mean Phe concentration was 502 ± 150 μmol/L during the past 6 months. Blood Phe concentrations were within the target range one week before the study (120 – 600 μmol/L). The 6 healthy individuals (males and females) had a mean age of 32 ± 4 years with a normal height (mean Z score -0.1 ± 1), body weight (mean 67 kg ± 14) and BMI (mean 23 ± 3 kg · m⁻²) (Table 1).

All participants were in good clinical condition at the time of the study and free of concomitant disease and their body weight was stable within the past 6 months. Participants were asked to keep a record of their entire food and beverage intake for 3 days. From this 3-day period the mean 24 hours energy intake was calculated. Participants were excluded when their normal level of exercise resulted in an energy requirement >25% above mean RDA for energy, as protein requirements under extreme physical activity have not been defined.

The nature, purpose, and potential risks of the study were explained to all subjects before they gave their written informed consent to participate. The Medical Ethical Committee of the University Medical Center Groningen approved the study protocol.

Materials
Isotopes: L-[1-¹³C]-valine and NaH¹³CO₃, both with enrichment over 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 pharmacy the afternoon before the study day and were used within 24 hours after preparation.
Table 1 Clinical characteristics of PKU patients and healthy controls studied for whole body protein metabolism

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>PKU patients</th>
<th>Healthy volunteers</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y)</td>
<td>27 (± 7)</td>
<td>32 (± 4)</td>
</tr>
<tr>
<td>Sex (M/F)</td>
<td>3/3</td>
<td>2/4</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>70 (± 9)</td>
<td>67 (± 14)</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>23 (± 3)</td>
<td>23 (± 3)</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>172 (± 3)</td>
<td>172 (± 9)</td>
</tr>
<tr>
<td>Height [Z score]</td>
<td>-0.6 (± 1)</td>
<td>-0.1 (± 1)</td>
</tr>
<tr>
<td>Energy intake (kcal · kg⁻¹ · day⁻¹)</td>
<td>40 (± 14)</td>
<td>38 (± 7)</td>
</tr>
<tr>
<td>Protein intake (g · kg⁻¹ · day⁻¹)</td>
<td>1.1 (± 0.1)</td>
<td>1.2 (± 0.1)</td>
</tr>
<tr>
<td>Phe tolerance at 5 y (mg Phe · kg⁻¹ · day⁻¹)</td>
<td>21 (± 9)</td>
<td></td>
</tr>
<tr>
<td>Phe tolerance at test (mg Phe · kg⁻¹ · day⁻¹)</td>
<td>11 (± 4)</td>
<td></td>
</tr>
<tr>
<td>Phe concentration last 2 years (μmol/L)</td>
<td>522 (± 106)</td>
<td></td>
</tr>
<tr>
<td>Phe concentration in plasma (μmol/L)</td>
<td>443 (± 100)</td>
<td>49 (± 10)</td>
</tr>
<tr>
<td>Tyr concentration in plasma (μmol/L)</td>
<td>77 (± 9)</td>
<td>67 (± 14)</td>
</tr>
<tr>
<td>Val concentration in plasma (μmol/L)</td>
<td>222 (± 42)</td>
<td>193 (± 52)</td>
</tr>
<tr>
<td>Albumine g/L</td>
<td>43 (± 2)</td>
<td>45 (± 2)</td>
</tr>
<tr>
<td>Total protein g/L</td>
<td>70 (± 3)</td>
<td>71 (± 3)</td>
</tr>
<tr>
<td>Urea mmol/L</td>
<td>4 (± 1)</td>
<td>5 (± 1)</td>
</tr>
<tr>
<td>Creatinine (μmol/L)</td>
<td>81 (± 12)</td>
<td>85 (± 5)</td>
</tr>
<tr>
<td>ASAT U/L</td>
<td>31 (± 5)</td>
<td>53 (± 8)</td>
</tr>
<tr>
<td>ALAT U/L</td>
<td>18 (± 7)</td>
<td>19 (± 11)</td>
</tr>
</tbody>
</table>

*All values mean ± SD

Diet:
For the PKU patients the protein intake conform the RDA advise (0.8 g protein · kg⁻¹ · d⁻¹) was composed out of amino acid mixture (PKU 3® Milupa, Friedrichsdorf, Germany) and the individual Phe tolerance as natural protein (0.1- 0.2 g protein · kg⁻¹ · d⁻¹). Patients used to another amino acid supplement were changed at least two weeks before the study to PKU 3®.

The protein intake for the healthy individuals conform the RDA advise (0.8 g protein · kg⁻¹ · d⁻¹) was composed out of 67% milk protein (high quality) and 33% vegetable protein (low quality).

On the test day meals for both groups consisted of liquid meal portions, fruit and biscuits. For PKU patients the liquid meal portions were composed of water, amino-acid mixture PKU 3® (Milupa, Friedrichsdorf, Germany), fat and malto-dextrin modules (Solagen® and Fantomalt®- Nutricia- Zoetermeer, The Netherlands) and an artificial flavor (®Flavour Sachet -SHS, Liverpool, United Kingdom). For healthy individuals the liquid meal portions were composed of milk, a milk protein module (Protifar plus®- Nutricia, Zoetermeer, The Netherlands), fat and malto-dextrin preparations (Solagen® and Fantomalt®). The valine content of milk protein was 6.3%, of wheat protein 4.5% and of protein of fruit 3.6%. The valine content of the amino acid supplement PKU
3% was 7.9%. This resulted in an average valine intake of 78 ± 5 µmol·kg⁻¹·h⁻¹ in PKU patients and 56 ± 1 µmol·kg⁻¹·h⁻¹ in healthy controls for the 4 h prandial period.

**Experimental Design**

The adaptation period of the test diet with 0.8 g protein·kg⁻¹·d⁻¹ (described as above) was 1 day, in which the intake was divided over 3 main meals and 3 snacks. The energy intake during the adaptation day was equal in both groups to the computed individual intake of the 3 days records, 40 (± 14) kcal·kg⁻¹·day⁻¹ for the PKU patients and 38 (± 7) kcal·kg⁻¹·day⁻¹ for the control group. The last snack was taken between 8 and 10 PM. After 10.00 PM no food or beverages aside from water were allowed. The study started at 08.00 AM after overnight fasting in the preprandial state. The first meal on the study day at 12.00 AM contained one third of the daily individually calculated energy and protein intake. After that, hourly meals provided one twelfth of the energy and protein intake. The energy intake for both groups was based on the individual’s 3 day food record. Calculation of the records was done by a dietitian (MvR) with the ZIS-food calculation computer program based on NEVO²⁴. A schematic diagram of the study day is shown in Figure 1.

Individuals were admitted to the Hospital Research Unit at 7:45 AM. An intravenous catheter was inserted into a vein for blood sampling, and another was placed in the opposite arm for the infusion of the labeled materials. Subsequently, baseline breath and blood samples were taken. The NaH¹³CO₃ infusion started at 8:00 AM. During the 1st hour, whole body NaH¹³CO₃ production was measured using a primed constant infusion of NaH¹³CO₃ (5 µmol·kg⁻¹ bolus followed by a continuous infusion of 5 µmol·kg⁻¹·h⁻¹). Four breath samples were taken from 15 to 60 min after the start of the NaH¹³CO₃ infusion at 15-min intervals. The NaH¹³CO₃ infusion was discontinued immediately after

Figure 1. Study protocol for whole-body L[1-¹³C]valine kinetics in PKU patients and controls preprandial and during hourly meals.
the last breath sample was taken, and replaced by L-[1-13C]-valine infusion bolus of 7.5 µmol·kg⁻¹ followed by a continuous infusion of 7.5 µmol·kg⁻¹·h⁻¹ for the next 7 h. Blood and breath samples were taken simultaneously every half hour for the first 2 h after the start of the L-[1-13C]-valine infusion. During the third hour, blood and breath samples were taken every 15 min. At 12:00 AM, the meal period was started by consumption of the first meal and continued for 4 h by consumption of a meal every 60 min. After the start of the meal period blood and breath samples were taken every 30 min for 3 hr and during the last hour samples were taken every 15 minutes. Amino acids, insulin and glucose were determined at start, at the end of the fasting period and at the end of the meal period. Total protein, albumin concentrations, platelet counts, liver enzymes, urea and creatinine were determined at the start of the test day by standard clinical chemistry methods. Urine was collected during the 24 hour adaptation and during the study in 2 periods of 4 hours (preprandial and prandial). Urine samples of these three periods were taken to determine urea and creatinine by standard clinical chemistry methods.

**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. 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. Phenylalanine concentrations in bloodspot (1 week before the testing day) were measured by the AccQ Tag method using high performance liquid chromatography according to the manufacturer’s protocols (Waters, Breda, The Netherlands). Analysis of all amino acid concentrations in plasma at the testing day were measured on a Biochrom 20 amino acid analyzer with the ninhydrine- method, according to manufacturer’s protocols (Biochrom, Cambridge, United Kingdom).

Measurement of 13CO2 isotopic enrichment was performed by sampling directly the glass container with a Heliview (MediChems, Seoul, Korea) continuous-flow isotope ratio-mass spectrometer as described by Vonk et al. L-[1-13C]-ketoisovaleric acid (KIVA) isotopic enrichment was determined according to Kulik et al. In short, standards with a tracer mole ratio for L-[1-13C]-KIVA ranging from 0 to 22% were prepared by enzymatic conversion with L-amino acid oxidase type 1 of standard mixtures of L-[1-13C]-valine with natural valine, as described earlier. Standards of L-[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 performed by gas chromatography coupled with mass spectrometry (GC-MS). The mass detector was a quadropole mass spectrometer (Finnigan Trace-MS Plus; Thermoquest-Interscience, Breda, Netherlands) used in electron impact mode. The gas chromatograph was fitted with a capillary column (J&W Scientific DB-1701, length 20 m, internal diameter 0.18 mm, film thickness 0.40 µm; Alttech, Netherlands). The mass spectrometer was operated in the selected ion-monitoring mode recording fragments at m/z 245 and 246 of unlabeled KIVA and L-[1-13C]-KIVA, respectively. All
isotopic enrichments were calculated against standard calibration curves.

**Evaluation of Primary Data**

The whole body rate of appearance of valine \( (R_a) \) was calculated at isotopic steady state using the inverted pool model described by Matthews et al for leucine kinetics\(^{28}\). When this isotopic model is applied to L-[1-\(^{13}\)C]-valine, enrichment of plasma L-[1-\(^{13}\)C]-KIVA is assumed to provide an adequate estimate of intracellular enrichment of valine\(^{26}\). The \( R_a \) (μmol valine kg\(^{-1}\) h\(^{-1}\)) was calculated according to the following equation:

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

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

The rate of oxidation of valine was calculated following the approach used by Van Goudoever et al. and described by Veeneman et al\(^{29,30}\). We did not use indirect calorimetry in our study to determine CO\(_2\) production as a measure of whole body bicarbonate production. In the approach of Van Goudoever et al., whole body bicarbonate flux is estimated before the L-[1-\(^{13}\)C]-valine infusion using a primed continuous infusion of NaH\(^{13}\)CO\(_3\) of short duration\(^{30}\). The NaH\(^{13}\)CO\(_3\) production \( (i_{\text{bic}}(\text{V})) \) from L-[1-\(^{13}\)C]-valine during valine infusion \( (i(\text{V})) \) was calculated according to:

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

Where \( \text{IECO}_2(\text{B}) \) 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, \( \text{IECO}_2(\text{V}) \) is the isotopic enrichment in APE of \(^{13}\)CO\(_2\) in expired air at isotopic steady state during the L-[1-\(^{13}\)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:

\[
\text{Ox} = i_{\text{bic}}(\text{V}) \times \left[ 100/\text{MPE(KIVA)} \right] \quad (\mu\text{mol valine kg}^{-1}\text{ h}^{-1})
\]

In this way, the oxidation rate of L-[1-\(^{13}\)C]-valine could be calculated without measuring VCO\(_2\).

In the preprandial period:

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

where \( \text{O (preprandial)} \) is the oxidation rate in the preprandial period.

During the meal period, recovery of labeled CO\(_2\) will be increased in comparison with preprandial. Estimates from the literature have been used, i.e. 0.74 ± 7 to 0.84 ± 8 preprandial and during and meal intake, respectively\(^{31}\). This represents an average 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.
Calculation of Whole Body Protein Metabolism

In Figure 2 the isotopic model for whole body protein metabolism is shown in a schematic diagram. In this model, influx of valine comes from whole body protein breakdown (B) and, when appropriate, from dietary intake (I) as described by Veeneman et al. Valine leaves the plasma amino acid pool by whole body protein synthesis (S) and oxidation (O). The input fluxes in this model result in label dilution of infused L-[1-\textsuperscript{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 R\textsubscript{a} 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.

The R\textsubscript{a} of dietary valine participating in whole body protein metabolism was calculated starting from the dietary intake of valine (I). This rate was multiplied by 0.8 to correct for first pass metabolism\textsuperscript{32,33}. Subsequently, this rate was corrected for the enlargement of the plasma valine pool resulting in the flux of dietary valine participating in whole body protein metabolism (J\textsubscript{I}). The amount of dietary valine entrapped in the enlarged pool size of valine (∆Q) was calculated by multiplying the increase in valine concentration with 20% splanchnic metabolism.

\[ O \text{ (prandial)} = \frac{O}{1.13} \]

Figure 2. The isotopic model of whole-body protein metabolism during preprandial and prandial period

The fluxes considered in this model shown are whole-body protein breakdown (B), synthesis (S) and oxidation (O) after correction for splanchnic metabolism (0.8 I) and the flux associated with the plasma amino acid pool enlargement (J (∆Q), infusion of \textsuperscript{13}C-valine (I), the flux (J\textsubscript{I}) associated with dietary intake of valine (I).
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in plasma by total body water, defined as 60% of body weight in these patients. 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. In a previous study, a continuous increase of plasma valine concentration during dietary protein intake was observed during the study period. This increase was assumed to be linear in time and the flux associated with the enlargement of the pool size of valine (JΔQ) to be constant. Accordingly, the total \( R_a \) of valine comprises the \( R_a \) of valine released from whole body protein breakdown, infusion of L-[1-\(^{13}\)C]-valine (i), and during the meal period the flux associated with dietary valine (J). At steady state, the \( R_a \) of valine equals the rate of disappearance (\( R_d \)) of valine. The total rate of disappearance of valine comprises whole body protein synthesis and oxidation. At steady state:

\[
R_a = B + J + i = S + O = R_d \text{ (\text{\( \mu \text{mol valine} \cdot \text{kg}^{-1} \cdot \text{h}^{-1} \))}
\]

This results in the following calculations.
During fasting:
\( B = R_a \text{ i} \)
and
\( S = R_a - O \text{ (preprandial)} \)
After meal:
\( B = R_a \text{ i} - J \)
and:
\( S = R_a - O \text{ (prandial)} \)
Protein balance was calculated by subtracting protein breakdown from protein synthesis.

Statistics
All values are given as means ± SD. Statistical analysis was done using Excell 2000 Microsoft computer program. The changes in protein metabolism in the preprandial and prandial states were compared using a paired 2-tailed Student’s \( t \)-test. Differences between the two groups (PKU patients and controls) in protein metabolism parameters were tested using the 2-tailed unpaired Student’s \( t \)-test. Statistical significance was assumed at \( P < 0.05 \).

RESULTS
The demographic and clinical details of the studied PKU patients and the healthy controls are given in Table 1. Both groups were comparable in demographics and base line laboratory values (albumine, total protein, platelet counts, transaminases, urea, creatinine). Excretion of urea and creatinine in urine was also within the normal range.

Primary data on concentrations and isotopic enrichments
Preprandial and prandial concentrations of glucose, insulin and amino acids for both healthy adults and adult PKU patients are shown in Table 2. Analysis of glucose and insulin
showed the expected insulin response in the meal period, which tended to be higher in the PKU group without reaching statistical significance. The response to the meals of the concentrations of amino acids varies depending on the amino acid considered. In the control group the rise in proline, alanine and valine concentration and the fall in ornithine concentration in the meal period were significant. In the PKU group the rise in proline, valine, isoleucine and leucine concentration and the fall in asparagine and citrulline concentration were significant in the meal period. Differences in plasma amino acid concentrations between the two groups in the preprandial period were statistically significant for phenylalanine and cystine. Plasma concentrations at the end of the meal period of valine, isoleucine, leucine, phenylalanine and lysine were

<table>
<thead>
<tr>
<th>Amino Acid</th>
<th>Controls At start</th>
<th>Controls At the end</th>
<th>PKU At start</th>
<th>PKU At the end</th>
</tr>
</thead>
<tbody>
<tr>
<td>Asp</td>
<td>16 ± 2</td>
<td>15 ± 2</td>
<td>15 ± 2</td>
<td>14 ± 3</td>
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<tr>
<td>Thr</td>
<td>120 ± 29</td>
<td>111 ± 16</td>
<td>132 ± 18</td>
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<tr>
<td>Ser</td>
<td>112 ± 19</td>
<td>110 ± 15</td>
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<tr>
<td>Asn</td>
<td>47 ± 11</td>
<td>46 ± 6</td>
<td>38 ± 7</td>
<td>25 ± 6†</td>
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<td>Glu</td>
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<td>67 ± 23</td>
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<td>Gln</td>
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<td>422 ± 60</td>
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<td>Pro</td>
<td>163 ± 66</td>
<td>248 ± 57†</td>
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<td>Gly</td>
<td>205 ± 34</td>
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<td>254 ± 53</td>
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</tr>
<tr>
<td>Arg</td>
<td>39 ± 5</td>
<td>38 ± 8</td>
<td>28 ± 17</td>
<td>49 ± 12†</td>
</tr>
<tr>
<td>Ess AA†</td>
<td>744 ± 61</td>
<td>770 ± 70</td>
<td>783 ± 69</td>
<td>1021 ± 99</td>
</tr>
<tr>
<td>Non ess AA‡</td>
<td>1425 ± 126</td>
<td>1585 ± 142</td>
<td>1512 ± 140</td>
<td>1631 ± 151</td>
</tr>
</tbody>
</table>

| Glucose    | 4 ± 1             | 4 ± 1              | 4 ± 1        | 5 ± 1‡        |
| Insuline   | 7 ± 2             | 76 ± 29†           | 9 ± 3        | 118 ± 49†     |

*All values mean ± SD, amino acid concentrations in µmol/L, glucose in mmol/L, insuline mE/L
† P <0.05 2-tailed paired Student’s t-test between preprandial and prandial
‡ P <0.05 2-tailed unpaired Student’s t-test between PKU and control at start (preprandial)
§ P <0.05 2-tailed unpaired Student’s t-test between PKU and control at the end (prandial)
† Ess AA: total essential amino acids minus Phe: Thr, Val, Met, Ile, Leu, Lys, His
‡ Non ess AA: total non essential amino acids minus Tyr: Asp, Ser, Asn, Glu, Gln, Pro, Gly, Ala, Citr, Cys, Arg, Orn
significantly higher in PKU patients than in controls.

In Figure 3 and Figure 4 the time courses are shown of $^{13}$CO$_2$ enrichment in expired air and $^{13}$C KIVA enrichment respectively, in plasma for both groups. As is clear from Figure 3, isotopic steady state of $^{13}$CO$_2$ in expired air during the NaH$^{13}$CO$_3$ infusion was reached between 30 and 60 min after the start of the infusion. The majority of the individuals reached isotopic steady state for $^{13}$CO$_2$ and $^{13}$C-KIVA between 180 and 240 min during the preprandial period and between 390 and 480 min during the prandial period. However some individuals showed a tendency to a small decrease at 180 minutes. The values of isotopic enrichment obtained during these time periods were used to calculate the steady-state valine fluxes reflecting whole body protein metabolism.

**Parameters of whole body protein metabolism**

In Table 3 the primary data are given of the valine fluxes in control subjects and PKU patients during both the preprandial and the prandial period. Rate of appearance of valine is not different between both groups, both before and after meals. The higher oxidation rate during the prandial period compared to the preprandial period reached significance only in the PKU group ($P < 0.01$ for the PKU group and 0.07 for the controls). During the prandial period, the resulting $R_a$ of dietary valine into the peripheral circulation was $42 \pm 4$ in the control group and $51 \pm 8 \mu$mol valine $\cdot$kg$^{-1}$ $\cdot$h$^{-1}$ in the PKU group ($P < 0.02$).

**Figure 3.** Breath $^{13}$CO$_2$ enrichment for the control group and the PKU group

- open circles for the control group
- closed circles for the PKU group
- $\Delta$: meal with 2/3 energy and protein
- $\Delta$: meal with 1/12 energy and protein
In Figure 5 whole body protein breakdown, synthesis and net protein balance during the preprandial and the prandial period are shown. As can be seen from this figure, the meal diminished whole body protein breakdown in both groups to a similar extent. Whole body protein synthesis was not influenced by the meal. Collectively, this changed the net protein balance from protein loss during the preprandial period (-21...
Protein metabolism in adult patients with Phenylketonuria

\[ 4 \pm 2 \text{ vs. } -17 \pm 6 \mu\text{mol valine} \cdot \text{kg}^{-1} \cdot \text{h}^{-1} \text{ control vs. PKU, NS} \] into protein accretion during the prandial period. \((16 \pm 9 \text{ vs. } 23 \pm 8 \mu\text{mol valine} \cdot \text{kg}^{-1} \cdot \text{h}^{-1} \text{ control vs. PKU, NS})\). In both groups the effect of the meal on net protein balance was significant \((P < 0.01)\).

Figure 5. Protein Breakdown, Synthesis and Balance, for control subjects and PKU patients during both preprandial and prandial.
DISCUSSION

The most important findings of this study were that turnover, oxidation, and net balance of L-[1-13C]-valine in adult PKU patients were comparable to the healthy adult individuals, while both groups received a protein intake at RDA level in the prandial period with frequent meals. Before discussing the results, some methodological issues should be addressed.

First, L-[1-13C]-leucine has been applied in many studies to trace whole body protein metabolism, however the use of L-[1-13C]-valine as tracer has advantages over [1-13C]-leucine metabolically. It has been reported that leucine compared to [1-13C]-valine shows a larger insulinomimetic effect on protein metabolism35;36. In our laboratory L-[1-13C]-leucine has been used previously in the study of whole body protein metabolism29;37-39. At doses normally used in the study of whole body protein metabolism, valine and leucine give similar values of the fluxes of protein breakdown, synthesis, and oxidation40.

Second, in general protein can be given as natural protein, as hydrolysate, and as free amino acids. In the studied healthy individuals the intake was given as natural protein conform the RDA advise (0.8 g protein · kg⁻¹ · d⁻¹) and was composed out of 67% milk protein (high quality) and 33% vegetable protein (low quality). In the PKU patients, the protein intake was given almost exclusively as amino acids. Free amino acids harbor differences in biological value and in absorption rates when compared to natural protein18;41-44. In RDA an adjustment is proposed of approximately 20% to compensate both for losses due to digestibility and protein quality for mainly vegetarian diets. In line with this we used an incremental factor of 1.2 to compensate for using amino acids instead of natural protein.

Third, the differences in Phe tolerance (range 6-15 mg Phe · kg⁻¹ · d⁻¹) in the studied PKU group resulted in small differences in the intake of Phe in the PKU group (range 4-10 mg Phe · kg⁻¹ · d⁻¹) in the meal period (t 240- t 480). However in all patients only marginally lower concentrations of Phe were found in the meal period compared to the preprandial period. This is in line with previous studies and shows that we measured in a rather stable situation of Phe metabolism45;46.

Further discussing the result we see that in response to the meals the concentrations of especially the branched chain amino acids in blood showed a larger increase in the PKU patients compared to the controls (Table 1). This effect is most likely due to the effect of the free amino acids consumed by the PKU patients and the higher concentrations of essential amino acids in the mixture compared to the natural protein taken consumed by the healthy controls (Table 3). Although a tendency of larger increases of insulin in the PKU group during the meals was observed, this tendency did not result in statistically significant differences of insulin, protein turnover and synthesis in the prandial period between healthy controls and PKU patients (Figure 5).

The enrichment of both 13CO₂ in expired air and 13C KIVA in plasma, reached at the end of both studied periods enabled us to calculate the steady-state valine fluxes reflecting whole body protein metabolism. These calculations showed that the increase in oxidation rate during the meal period was the only parameter that reached statistical significance in the PKU group, where the differences between the
two groups preprandial and prandial were not significantly different (Table 3). The results of this study showed that turnover, oxidation, and net balance of L-[1-13C]-valine in PKU patients were comparable to the control group of healthy individuals both during the preprandial and prandial period. Very limited data are available on whole body protein metabolism in PKU patients. Thompson et al studied whole body protein metabolism in PKU patients at high plasma Phe concentrations under preprandial conditions using a primed continuous infusion of L-[1-13C]-leucine, and showed that the protein turnover was comparable to healthy individuals47. Notwithstanding that we studied patients at lower plasma Phe concentration, the results of the preprandial period in our study were comparable with the results of Thompson et al47. Together with the findings of the present study during meals the studies strongly suggest that protein turnover in PKU patients is comparable with healthy controls under these study conditions. This conclusion, however, necessitates a restriction. The results are achieved under the strict conditions of continuous nutrition to achieve steady state as in the studies of Veeneman et al29;38. However, in PKU it has been shown that unequal distribution of amino acids and natural protein might be hypothesised to have specific influences on the use of phenylalanine and other amino acids16;46;48-50. Further studies under less ideal nutritional conditions are warranted to test the hypothesis for day to day care.

CONCLUSION

The results of the present study show that there is no difference in valine turnover, oxidation and net protein balance between healthy adults and PKU patients. Therefore, these data do not support the recommendation of a protein intake in PKU patients higher than RDA as suggested in the guidelines of the Medical Research Council and the Ross Metabolic Nutrition Support System and in studies in PKU children51;52. Knowing that protein metabolism is normal in PKU patients under the present ideal study conditions, further studies are necessary to investigate whole protein turnover under less ideal nutritional conditions as practiced in daily life.

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