The 24-h whole body leucine and urea kinetics at normal and high protein intakes with exercise in healthy adults

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Forslund, Anders H., Leif Hambraeus, Roger M. Olsson, Antoine E. El-Khoury, Yong-Ming Yu, and Vernon R. Young. The 24-h whole body leucine and urea kinetics at normal and high protein intakes with exercise in healthy adults. Am. J. Physiol. 275 (Endocrinol. Metab. 38): E310–E320, 1998.—In healthy adult men adapted to a diet/exercise regimen for 6 days, the effects of small, frequent meals supplying daily protein intakes of 1 (n = 8) or 2.5 g·kg−1·day−1 (n = 6) on leucine oxidation, urea production, and whole body protein synthesis (PS) and degradation (PD) have been compared with the use of a 24-h continuous intravenous [13C]leucine and [15N,15N]urea infusion protocol. Two 90-min periods of exercise (~50% maximal O2 consumption) were included during the fasting and the fed periods of the 24-h day. Subjects were determined to be at approximate energy, nitrogen, and leucine balances on both diets. Increased protein intake raised the urea production rate; the absolute rate of urea hydrolysis was the same on both diets. When the first-pass splanchnic uptake of leucine was taken to be 25% of intake, PS was stimulated by feeding (after an overnight fast) at both protein intake levels (P < 0.05 and P < 0.01), whereas PD declined significantly (P < 0.01) at both protein levels. Protein gain at a high protein intake appears to be the result of both a stimulation of PS and a marked decline in PD, whereas at a less generous intake, the gain appears to be a result of a fall in PD with a less evident change in PS. Exercise moderately decreased PS during and/or immediately after exercise at each protein level, and there was a postexercise-induced increase (P < 0.01) in PD, which was more dramatic when feeding was at the higher protein intake level.

protein synthesis; protein degradation; urea production; urea hydrolysis; leucine oxidation; homeostasis; nitrogen balance; leucine balance; amino acids; energy balance

THE CONTENT OF BODY protein, which is relatively constant from day to day throughout most of adult life, although it declines slowly over a longer time frame (19, 36) in the face of wide variations in the dietary intake of protein, is achieved via a complex series of changes in rates of whole body protein turnover, amino acid oxidation, urea production, and nitrogen (N) excretion during the fasting, fed, and postprandial periods of the 24-h day (33, 34, 35, 42, 44, 45). Furthermore, there are potentially substantial gains and losses of body proteins that occur during the diurnal cycle of feeding and fasting (34, 35), the extent of which appears to depend on the level of dietary protein intake (37). Gibson et al. (23) have examined the impact of low and high protein intake levels (2 and 14% of energy intake, respectively) on leucine kinetics (as an index of whole body protein turnover) and found that there was a greater stimulation of protein synthesis after the high vs. lower protein meals. The present study, which involved a different experimental design, extends these earlier findings through an analysis of the dynamics of whole body leucine metabolism in subjects given a constant intravenous tracer infusion of [1-13C]leucine for 24 h (13).

An additional although secondary purpose of this study was to examine 1) how physical activity influences body protein balance and metabolism (16, 19) within a range of adequate dietary protein intake levels and 2) the proposition by Jackson (28–30) that urea hydrolysis in the gut is regulated and that this aspect of urea metabolism plays a key role in the achievement and maintenance of body N balance at normal protein intakes. These investigators have concluded that the rate of urea production within a wide range of adequate protein intakes, from above ~30 g protein/day, in adults is relatively constant (28).

In the present study, the status of whole body protein turnover and urea metabolism was probed with the aid of [13C]leucine and [15N,15N]urea tracers given continuously for 24 h in healthy adults receiving one of two different but adequate protein intakes: a so-called “normal” intake, equivalent to 1 g protein·kg−1·day−1, and a “high” intake of 2.5 g protein·kg−1·day−1 (8 and 21% of total energy intake, respectively). This study is composed of a unit of our more elaborate investigation of the effects of diet and exercise on macronutrient metabolism and body composition (13, 20). Some of the data for subjects given the normal protein intake presented here have been reported, in part, previously (13). However, our purpose is to present a more detailed account of our earlier findings combined with new data obtained under essentially the same experimental conditions, except for the new subjects having been given a high protein diet before undertaking the tracer kinetic measurements.

METHODS

Subjects

Two groups (n = 8 and n = 6) of healthy male volunteers participated in the study. Detailed descriptive data for these subjects are summarized in Table 1. The subjects were recruited from the population of students and employees at Uppsala University. All were physically fit but not competitive athletes, and they were in good health as determined by medical history and physical examination; none of them smoked or had excessive alcohol consumption. All subjects gave their written informed consent, and the study was...
and physical exercise was performed on a cycle ergometer 1–5 at the metabolic unit of the Uppsala University Depart- ment of Uppsala University. Experimental Design

Values are means ± SD. Body fat was calculated as previously stated (13). \( \text{V}O_{2\text{max}}, \) maximal \( \text{O}_2 \) consumption; \( \text{TBW} \), total body water; BMR, basal metabolic rate.

approved by the Ethical Committee of the Faculty of Medicine of Uppsala University.

Experimental Design

Subjects were studied on an outpatient basis during days 1–5 at the metabolic unit of the Uppsala University Department of Nutrition. They ate the standardized diet for 7 days, and physical exercise was performed on a cycle ergometer within the metabolic unit. Day 6 was used as a so-called \"sham infusion\" day, and air samples were taken once every hour to determine the background output of \( ^{13}\text{CO}_2 \) in expired air (13). \( \text{O}_2 \) uptake and \( \text{CO}_2 \) output (indirect calorimetry) were recorded at regular intervals throughout the day. In the evening of day 6, the subjects were dressed in the direct calorimeter suit and intravenous catheters were inserted in the veins on the dorsal side of both hands, as previously described (13). Physiological saline was slowly infused during the night until the start of the tracer infusion at 0600 on day 7. Day 7 was the tracer infusion day, and the primed, continuous 24-h intravenous infusion of stable isotopic tracers was started at 0600 and continued until 0600 on day 8. The cycling was performed between 0830 and 1000 and between 1600 and 1730. When not cycling, subjects usually sat in a chair while watching a video or reading.

Physical Capacity and Workload

During the study, the subjects underwent a physical activity program, the level of which was set at 45–50% of maximal \( \text{O}_2 \) consumption, corresponding to an external workload of 110 ± 13 W. On each day of the study, including the day of the tracer experiment, the program of exercise included two 90-min periods, with one carried out in the morning when subjects were fasting and the other in the afternoon when the subjects were in the fed state. Details have been presented previously (13).

Diet

The nutrient content of the diet was estimated using software (MATs version 3.0, Rudans Data, Vasteras, Sweden) based on the Swedish nutrient database from National Food Administration (PC-diet, version 2-93, Uppsala, Sweden). Basal metabolic rate was calculated from the Food and Agriculture Organization/World Health Organization/United Nations University equations (17) on the basis of the body weight of the subject, which was measured after an overnight fast at ~1 wk or less before the experiment began. For total energy expenditure, not including the experimental program of physical exercise, a physical activity level (17) of 1.55 was used during days 1–6 and a factor of 1.27 was used during day 7 (infusion day) because subjects were sedentary except during the 90-min exercise periods, then adding the extra energy spent for physical exercise. The diet was designed to keep the subject in energy balance and to supply either 1 or 2.5 g protein·kg\(^{-1}\)·day\(^{-1}\), using a drink based on milk as the principal protein source. The drink was flavored with raspberry or banana. For the lower protein diet, specially prepared protein-free cookies were used as an additional energy source to balance energy expenditure. They were baked with a protein-rich mix (low protein and milk-free mix; Semper, Stockholm, Sweden), beet sugar, margarine, and sunflower oil and flavored with raisins or chocolate. They contained 0.3% energy from protein, 46% energy from fat, and 54% energy from carbohydrate. The fat-to-carbohydrate energy ratio in the nonprotein energy was 40:60. For the higher protein diet, the cookies supplied, as milk protein, ~40% of the total protein intake. The composition of the experimental diet is described elsewhere (13). During days 1–5, the food was given as a breakfast, lunch, and dinner, with two small meals in between. During days 6 and 7, the food was equally distributed as 10 hourly meals, from 1200 to 2100. Energy balance was confirmed both by direct and indirect calorimetry (13) as follows.

Rates of \( \text{O}_2 \) consumption and \( \text{CO}_2 \) production during the 24-h study were assessed using a modified ergiospirometer (Sensormedics 2900Z, Anaheim, CA), which allows for use of an open-hood technique for up to a work load of 200 W using an air flow rate of 270 l/min and permitting an \( \text{O}_2 \) uptake of ~3.0 l/min. The airflow rate was adjusted to keep the \( \text{CO}_2 \) concentration in expired air between 0.5 and 1.0%, which is the optimal concentration for the sensors according to the manufacturer. Autocalibration was performed every 30 min using two standard gases with known contents of \( \text{O}_2 \) and \( \text{CO}_2 \) (16% \( \text{O}_2-4\% \text{CO}_2 \) and 26% \( \text{O}_2-0\% \text{CO}_2 \) in N, respectively). Inspired air was checked every 10 min. Interpolations of \( \text{O}_2 \) and \( \text{CO}_2 \) were done during short periods (<15 min) while the subject was eating and every 8 h when a manual recalibration of the instrument was performed. The oxidation of protein was derived from the measurements of leucine oxidation, and the net utilization of fat and carbohydrate was calculated according to the equations of Garlick et al. (21). To determine the contribution made by the macronutrients to energy expenditure, the following constants were used: protein 4.4 kcal/g, fat 9.35 kcal/g, and carbohydrate 3.75 kcal/g. To convert kilocalories to joules, a factor of 4.186 was used. Energy turnover was calculated by adding the energy values from the various macronutrients together. A nonprotein respiratory quotient was calculated by assuming that 0.966 liter of \( \text{O}_2 \) was consumed and that 0.802 liter of \( \text{CO}_2 \) was produced per gram of protein oxidized (i.e., protein respiratory quotient = 0.83) (31). A 24-h direct calorimetry was continuously assessed using the suit calorimeter as previously described (25).

Twenty-Four-Hour Stable Isotope Trace Protocol

Details of the 24-h tracer protocol have been presented previously (13). In brief, primed, continuous 24-h intravenous infusions of stable isotope tracers were started at 0600 on day 7 and continued until 0600 on day 8. The L-[\( ^{13}\text{C} \)] leucine (99 atoms/100 atoms; MassTrace, Woburn, MA) was administered at a rate of ~8 \( \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{h}^{-1} \) (prime 12 \( \mu\text{mol/kg} \)). In

Table 1. Characteristics of subjects, predicted BMR, dietary intake, and energy balance at normal and high protein intakes

<table>
<thead>
<tr>
<th></th>
<th>Normal</th>
<th>High</th>
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<tbody>
<tr>
<td>Age, yr</td>
<td>27 ± 12.5</td>
<td>29 ± 14.7</td>
</tr>
<tr>
<td>Body weight, kg</td>
<td>77.5 ± 6.7</td>
<td>80 ± 11.7</td>
</tr>
<tr>
<td>Height, m</td>
<td>1.67 ± 0.06</td>
<td>1.86 ± 0.09</td>
</tr>
<tr>
<td>( \text{V}O_{2\text{max}}, \text{l/min} )</td>
<td>4.05 ± 0.5</td>
<td>3.76 ± 0.23</td>
</tr>
<tr>
<td>Body fat, %</td>
<td>16.5 ± 5.6</td>
<td>19.3 ± 5.1</td>
</tr>
<tr>
<td>Predicted TBW, liters</td>
<td>46.0 ± 2.5</td>
<td>40.1 ± 4.5</td>
</tr>
<tr>
<td>Predicted BMR, MJ/day</td>
<td>7.8 ± 0.4</td>
<td>7.8 ± 0.7</td>
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</table>

On day 7

| Energy intake, MJ/day | 16.1 ± 1.1 | 15.5 ± 0.9 |
| Protein, g/day | 78 ± 6.1 | 199.0 ± 28.7 |
| Fat, g/day | 158 ± 10.8 | 1283 ± 49 |
| Carbohydrate, g/day | 539 ± 38.7 | 434.0 ± 16.8 |
| Energy balance, MJ/day | 0.86 ± 0.84 | 0.73 ± 1.03 |

Details of the 24-h tracer protocol have been presented previously (13). In brief, primed, continuous 24-h intravenous infusions of stable isotope tracers were started at 0600 on day 7 and continued until 0600 on day 8. The L-[\( ^{13}\text{C} \)] leucine (99 atoms/100 atoms; MassTrace, Woburn, MA) was administered at a rate of ~8 \( \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{h}^{-1} \) (prime 12 \( \mu\text{mol/kg} \)). In
addition, a [13C]bicarbonate prime (1.5 µmol/kg) was given (99 atoms/100 atoms; Cambridge Isotope Laboratories, Andover, MA). [13N,15N]urea (98 atoms/100 atoms; Cambridge Isotope Laboratories) was infused at a rate of ~7 µmol·kg⁻¹·h⁻¹, with the tracer dissolved in the saline also used for leucine. The urea priming dose was 88 µmol/kg, given over 1 min. All tracers were tested for sterility and lack of pyrogenicity by independent laboratories (Microtest Laboratories, Agawam, MA, and Pharmaceutical Dept., Univ. Hospital, Uppsala, Sweden). One intravenous catheter was used for the gear-driven pump (model AS40A, Baxter Health Care, Deerfield, IL). The other catheter was used for blood sampling.

Urine collections were performed at consecutive intervals every 3 h to measure urinary urea N, nonurea N, and total N during the 24-h tracer day. Indirect calorimetry was virtually continuous throughout the 24-h period (except during meal ingestion or instrument calibration). Each blood sample, ~5 ml, was collected in sodium-heparin-coated glass tubes as previously described (13). Samples were then centrifuged at 1,200 g for 15 min at ~4°C. Then plasma was removed and stored in sealed aliquots at ~20°C until analysis. Blood sampling was every one-half hour; it was more frequent (every 15 min) during the exercise periods. Breath samples were taken at the same frequency as blood samples, except that only hourly breath samples were collected during the night (2300–0600) to reduce disturbance during sleep. The collection and analysis of expired air for 13CO₂ abundance have been described previously in detail (13). The collection of sweat and determination of sweat N losses were also performed as described previously (25).

Analyses

Analysis of total N in urine was performed using the micro-Kjeldahl method (Kjeltec Auto Analyzer model 1030, Perstorp Analytical, Tecator, Sollentuna, Sweden). Urea N analysis in urine was accomplished via an enzyme procedure (Granultest 15-Plus, Diagnostica-Merck, Darmstadt, Germany). Urine collection bottles contained 15 ml of 6 N HCl as preservative. Analyses of urea and N concentrations in 24-h sweat and plasma samples were performed using the above methods. Leucine concentration was determined in plasma using ion-exchange chromatography (LKB 4151 Alpha Plus amino acid analyzer, Pharmacia-LKB Biochrom, Cambridge, UK). Plasma [α-13C]ketoisocaproate ([13C]KIC) and plasma [13N,15N]urea abundances over baseline (mole fraction over baseline) were determined as described earlier in detail (13). Also, analyses of leucine and urea in the infusates were carried out at Massachusetts Institute of Technology as described earlier (13).

Calculations

Whole body leucine oxidation. Whole body leucine oxidation was computed as previously described (13, 14) using plasma [13C]KIC enrichment over baseline as an index of the precursor leucine pool enrichment. Before the plateau in plasma [13C]KIC enrichment had been reached during the first 90 min of the 24-h infusion, leucine oxidation during each of these initial one-half hourly intervals was assumed reasonably to be equal to the leucine oxidation rate during the first one-half hourly interval of the initial plateau period, which began at 90 min into the tracer protocol. Therefore, whole body leucine oxidation for the entire 24 h was directly measured for 45 of the total 48 one-half hourly intervals.

Measured 24-h leucine balance. Body leucine balance (input – output) was computed as follows

\[ \text{Input (µmol/kg)} = \text{dietary leucine + intravenous tracer} \]  (1)
\[ \text{Output (µmol/kg)} = \text{sum of determined whole body leucine oxidation for 48 one-half hourly intervals} \]  (2)

Leucine losses in sweat were shown (13) to be close to negligible (see also RESULTS), and so they were not included in the above equation.

Irreversible protein N loss derived using the leucine oxidation technique. This estimate of irreversible protein N loss (IPNL) was computed as follows

\[ \text{IPNL (mg protein · kg⁻¹ · day⁻¹)} = \text{[24-h leucine tracer]} - 24-h \text{ leucine tracer tracer given} \]  (3)

where 1) the 24-h leucine tracer given corrects for the additional oxidation of the nonmass loss leucine tracer under these conditions of generous leucine intakes. At intakes above the leucine requirement, the additional leucine given as tracer is presumed to be disposed of via oxidation. Hence this tracer input, which accounts for only a very small and insignificant fraction of the total N losses, should first be deducted from the total leucine oxidation before the IPNL is estimated. 2) The factor 100/8 assumes 8% (wt/wt) leucine in whole body proteins as previously described (14). 3) The factor 8/10.03 is an additional downward correction for the mismatch (in this study) between leucine in the diet (10%) and leucine in the body (8%). IPNL (mg protein · kg⁻¹ · day⁻¹) may be expressed in units of N (by dividing by 6.25). This approach to compute IPNL has been discussed in more detail previously (12, 13, 14).

IPNL using the N excretion technique. This second estimate of IPNL adds to the term “corrected 24-h urea N excretion + 24-h nonurea N excretion” a value of 8 mg N · kg⁻¹ · day⁻¹, as described previously (14), to account for unmeasured N losses (such as in feces) that would also reflect amino acid catabolism and for purposes of comparing the data with those based on the first method above. It is possible that the value of 8 mg N · kg⁻¹ · day⁻¹ would be somewhat higher for the high protein intake level, but in view of the good digestibility of milk proteins (17, 18), the factor would presumably remain below ~20 mg N · kg⁻¹ · day⁻¹. Thus, for the high protein diet, the IPNL would be slightly lower than it might actually be, and we consider this possible error in the results.

Total urea N production. We computed urea N production (UP) as previously described (13) for each 30-min interval also using the approach as for leucine oxidation during the first 90 min of the 24-h tracer period. Hence UP was measured over 45 of the 48 one-half hourly intervals. The mole fraction over baseline was used in the equation to compute total endogenous UP.

Twenty-four-hour urea N excretion. Urea N excretion was computed over 24 h exactly as previously described (13), including a correction for changes in body urea pool size. Total body water was predicted from the equations by Watson et al. (43). It should be noted that a number of subjects failed to void urine during each consecutive (every 3 h) interval. Therefore, because total body water may have changed between pre-, during, and postexercise, we computed urea excretion data over the entire 24-h time frame (and not for each interval every 3 h).

Twenty-four-hour urea N hydrolysis. The hydrolysis of urea N was computed as the difference between 24-h UP and 24-h UP.
urea N excretion and was expressed as percent UP as previously described (12).

Leucine flux and rates of leucine disappearance into protein synthesis and appearance from protein breakdown. For the fasting condition between 90 and 150 min and the fed condition between 540 and 600 min into the 24-h tracer infusion, plasma leucine flux and rates of nonoxidative leucine disappearance (i.e., protein synthesis) and leucine appearance via protein breakdown were calculated as previously described (15). Thus, for protein synthesis at these times, the estimation was based on leucine flux minus leucine oxidation, and for breakdown, it was based on the leucine flux minus leucine endogenous input (diet and/or tracer). For the fed condition, it is also necessary to take into account the splanchnic first-pass uptake of dietary leucine (6, 9, 10, 11, 26, 40) as well as its metabolic fates via protein synthesis, oxidation, or release into the peripheral circulation as α-ketoisocaproic acid. The latter rate is assumed to be relatively low (5), and therefore it can be ignored here.

Hence we have applied the approach described previously (15) to take this splanchnic effect into account. The factor used for the first-pass disappearance of absorbed leucine was taken to be 25% for both protein levels. It is possible that at the high protein intake, the percent first-pass disappearance would be less than this value of 25%, although there is insufficient published evidence to establish whether this is actually so. However, the lowest reported values based on tracer disappearance for the first-pass disappearance of absorbed leucine within the splanchnic region approximate 10% (9, 26). We will, therefore, include this value in the presentation of the RESULTS and the DISCUSSION.

We also assume that there is some oxidation of leucine that is taken up during its first pass through the splanchnic region (46), and so the fraction of the splanchnic disappearance of leucine oxidized was estimated from the present group mean data for the whole body leucine oxidation rates and leucine fluxes, also as previously discussed (15). These values for the 1- and 2.5-g protein intakes were 0.32 and 0.46, respectively. If these approximations are either too high or too low, then fed-state rates of whole body protein synthesis will have been under- or overestimated, respectively.

Therefore, for the fed state, the splanchnic uptake-corrected flux and rates of whole body protein synthesis and breakdown were calculated as follows, where a first-pass disappearance of 25% was assumed

$$f_c = \text{measured flux} + (I \times 0.25)$$

where $f_c$ is corrected plasma flux and I is dietary leucine intake. Then

Corrected oxidation for 1-g level ($\text{ox}_1$) = measured oxidation + [(I $\times$ 0.25) $\times$ 0.32]

Corrected oxidation for 2.5-g level ($\text{ox}_2$) = measured oxidation + [(I $\times$ 0.25) $\times$ 0.46]

Corrected protein synthesis = $f_c - \text{ox}_c$

Corrected protein breakdown = $f_c - (I + i)$

where I is tracer input. A discussion of this approach for estimating the fed-state rates of whole body protein synthesis and breakdown in comparison with those used by other investigators, as described recently, will be given below.

Finally, we also calculated rates of leucine appearance (protein breakdown) and disappearance (protein synthesis) during the relevant periods of observation identified below using the approach taken for non-steady-state conditions as proposed by Steele (39) and modified by Boirie et al. (7). Because the results obtained were essentially identical to those derived by the approach outlined above, we have not included these additional data in the RESULTS.

For purposes of analysis of the isotopic data, the fasting period was taken to be the 10th hour after the last meal, which was taken at 2100 on the previous day (or 90–150 min experimental time of the tracer protocol). The fed period, which lasted from 1200 to 2100, was taken to be 540–600 min into the tracer protocol. These times also corresponded to the preexercise periods (see Table 6). The exercise period was chosen as the last 60 min within each 90-min period, and the postexercise period corresponded to 300–360 min of experimental time in the case of the fasting phase and 750–810 min of experimental time for the fed, postexercise phase.

Statistical Analysis

For comparison of daily leucine kinetics, urea and N data, for the two diet groups, the Student's t-test was used. However, when one variable was measured (repeatedly) at different time frames (e.g., leucine oxidation before, during, and after exercise) within one physiological state (fasting or feeding), we performed a repeated-measures ANOVA followed by pair-wise comparison among means using the Student-Newman-Keuls test. To evaluate IPNL at each protein intake and as determined by the different methods, a one-way ANOVA was used. To compare a variable with a zero value (e.g., 24-h leucine balance), a two-tailed one-sample t-test was used. Data are presented as means ± SD. An α-level of 0.05 was considered to be statistically significant.

RESULTS

The 24-h pattern of $^{13}$C abundance (over baseline) in plasma $[^{13}$C]$\text{KIC}$ for the two diet groups is presented in Fig. 1. As expected, plasma $[^{13}$C]$\text{KIC}$ declined with exercise alone, possibly because of the relatively short exercise periods (90 min each) in relation to the large size and slow turnover of the body urea pool. Thus the daily rates of leucine oxidation and urea production and excretion, together with the body leucine and N balances, are summarized in Table 2 for each protein intake level. The daily leucine oxidation rate paralleled protein intake, but the group mean differences (leucine oxidation) did not exactly reflect the differences in leucine intake, leading to a tendency for a higher daily leucine balance at the 2.5-g protein level. Furthermore, the N balances did not differ significantly between the
two groups, and neither was significantly different from a zero or an equilibrium value. If the assumed unmeasured losses (see METHODS) of 8 mg N·kg⁻¹·day⁻¹ were higher for the high protein intake, then the mean N balance values of the two groups would have even been closer.

There was a general concordance between the leucine oxidation, protein intake, and N excretion data for the two dietary groups as the results for IPNL indicate (summarized in Table 3). Thus, from the leucine oxidation data, a mean "protein" output (in mg protein·kg⁻¹·day⁻¹) of 1,053 and 2,243 at the normal and high protein intakes, respectively, was predicted. These were not significantly different from determinations based on N excretion data for both diet groups or from the total protein intake in the normal protein group. There was a small but significant difference between IPNL estimates from N excretion and leucine oxidation vs. those predicted from protein intake for the high protein dietary level. This might be accounted for, at least in part, as a result of a slightly lower digestibility (which was not measured here) of dietary protein at the high vs. normal protein intake level. Thus the good agreement between these different values for IPNL reflects on the precision with which these kinetic/metabolic studies have been carried out and on the adequacy of the [¹³C]leucine oxidation model.

With respect to the method of calculation of IPNL from leucine oxidation, it is important to recognize that for a specific short time frame (within the 24-h period), there are uncertainties about the amino acid profile of proteins that are lost from the body. However, under conditions of neutral or close-to-neutral 24-h amino acid balance (input – output), the amino acid composition of the protein lost would be expected to be similar to that of the protein fed. Hence, as noted in METHODS,
use of the factor 100/8 alone would overestimate IPNL unless the downward correction is made for the concentration (wt/wt; %) of leucine in the diet given in these studies (~10%) (see METHODS).

The rate of urea production was approximately two-fold higher for the high vs. normal protein intake, and urea excretion was nearly threefold greater for the high vs. lower intake level (Table 2). The group mean urea N production rate was about equal to the N intake for the high protein group and exceeded intake by 18% at the normal intake of protein. Although the proportion of urea N that was hydrolyzed was 48% lower for the high vs. normal protein intake, the absolute rates of urea hydrolysis were essentially the same for the two diet groups.

The effects of fasting and feeding on whole body leucine kinetics at specific times during the 24-h day are summarized in Table 5. We present values here for "uncorrected" and those based on "corrected" estimates for the fed state, assuming either 25% or 10% first-pass disappearances of absorbed leucine for both diets. However, the comparisons that we will discuss are based on the former assumption, since we consider this to be more appropriate for reasons outlined in the DISCUSSION. Thus leucine fluxes increased with feeding, and this response was greater at the high protein intake level. A comparable pattern of change was observed for leucine oxidation. The mean fed-to-10th hour fast leucine oxidation ratios were 2.0 and 3.1 for high vs. normal protein intake, the absolute rates of urea hydrolysis were essentially the same for the two diet groups.

Loses of N, urea, and leucine via integumentary sweat were measured, and the results are summarized in Table 4. Mean urea N losses were moderately higher (although not statistically significant) for the high protein intake but still only accounted for a very small fraction of the loss that occurred via urine. Integumentary leucine losses were significantly higher for the generous vs. normal protein intake, but in relation to daily leucine oxidation, they were negligible.

Table 2. Leucine oxidation and balance, N balance, and urea N kinetics in healthy adults at normal and high protein intakes

<table>
<thead>
<tr>
<th></th>
<th>Normal (n = 8)</th>
<th>High (n = 6)</th>
<th>High/Normal (mean, %)</th>
</tr>
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<tbody>
<tr>
<td>Leu intake (total), mg·kg⁻¹·day⁻¹</td>
<td>127 ± 0.8</td>
<td>277 ± 0.2</td>
<td>218</td>
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<tr>
<td>Leu oxidation, mg·kg⁻¹·day⁻¹</td>
<td>131 ± 11</td>
<td>251 ± 18b</td>
<td>192</td>
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<tr>
<td>Leu balance, mg·kg⁻¹·day⁻¹</td>
<td>-4.6 ± 10.5c</td>
<td>25.7 ± 17.8d</td>
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<tr>
<td>N intake, mg N·kg⁻¹·day⁻¹</td>
<td>157</td>
<td>392</td>
<td>250</td>
</tr>
<tr>
<td>Total N excretion, mg N·kg⁻¹·day⁻¹</td>
<td>142 ± 39</td>
<td>369 ± 25b</td>
<td>260</td>
</tr>
<tr>
<td>N balance, mg N·kg⁻¹·day⁻¹</td>
<td>14 ± 39f</td>
<td>23 ± 25d</td>
<td>164</td>
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<tr>
<td>Urea N excretion, mg·kg⁻¹·day⁻¹</td>
<td>185 ± 28</td>
<td>386 ± 36b</td>
<td>209</td>
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<tr>
<td>Urea N hydrolysis, % of UP</td>
<td>101 ± 43</td>
<td>301 ± 49b</td>
<td>298</td>
</tr>
<tr>
<td>Hydrolyzed urea N, mg·kg⁻¹·day⁻¹</td>
<td>85 ± 46</td>
<td>84 ± 46c</td>
<td>99</td>
</tr>
</tbody>
</table>

Table 3. Estimates of IPNL from N excretion and from leucine oxidation at normal and high protein intakes in adults

<table>
<thead>
<tr>
<th></th>
<th>Normal</th>
<th>High</th>
</tr>
</thead>
<tbody>
<tr>
<td>IPNL</td>
<td>889 ± 246*</td>
<td>2,306 ± 154</td>
</tr>
<tr>
<td>From N excretion</td>
<td></td>
<td></td>
</tr>
<tr>
<td>From leucine oxidation</td>
<td>1,053 ± 113*</td>
<td>2,243 ± 177</td>
</tr>
<tr>
<td>Dietary protein intake</td>
<td>981*8</td>
<td>2,450±0</td>
</tr>
</tbody>
</table>

Irreversible protein N loss (IPNL) values are means ± SD in mg protein (N×6.25)·kg⁻¹·day⁻¹. *Differences within normal, P = 0.17 (NS; ANOVA). †Dietary intake is slightly higher than 2 other values (ANOVA) for high, P = 0.05.
Table 4. Twenty-four-hour sweat losses of total N, urea N and leucine after a six-day adaptation to diets containing normal or high amounts of protein

<table>
<thead>
<tr>
<th>Condition</th>
<th>Total N</th>
<th>Urea N</th>
<th>Leucine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>141.6 ± 46.1</td>
<td>92.9 ± 36.1</td>
<td>2.14 ± 1.21</td>
</tr>
<tr>
<td>High</td>
<td>169.6 ± 93.0*</td>
<td>120.3 ± 61.4*</td>
<td>12.8 ± 6.3†</td>
</tr>
</tbody>
</table>

Values are means ± SD in mg/day. Amounts of protein: normal, 1 g·kg⁻¹·day⁻¹; high, 2.5 g·kg⁻¹·day⁻¹. *NS vs. normal. †P < 0.01 vs. normal.

the 1- and 2.5-g groups, respectively (P < 0.01). Leucine oxidation at the 10th hour of fasting differed between the two protein levels (P < 0.01).

With the assumption of a 25% first-pass uptake of dietary leucine, whole body protein synthesis increased with feeding by about 34 and 10% for the high and normal protein groups, respectively (P < 0.05, P < 0.01). Protein degradation was reduced with feeding for both diets but more so when the high protein diet was fed (60% decline; P < 0.01). It also might be noted from Table 5 that an unrealistically low rate of whole body protein degradation was computed for the fed state when the plasma kinetic data were not adjusted for a first-pass disappearance of dietary leucine. Rates of whole body protein synthesis and degradation during the fasting (10th hour) period did not differ among the normal and high protein intakes, although the rates of leucine oxidation did differ as noted above.

The foregoing estimates of the changes in fed-state protein synthesis and breakdown were based on correction of the plasma fluxes for a 25% first-pass disappearance of absorbed leucine at both the normal and high protein intakes. It is possible that the percent disappearance at the high intake might have been lower than that at the normal intake, in which case a reasonable estimate might be 10% rather than 25% (9, 26). Hence, we also present in Table 5 corrected estimates of flux, protein synthesis, and breakdown by using a 10% estimate for both protein levels. As shown here, the stimulation of protein synthesis due to feeding would have been less and possibly not different between the two protein intake levels. Conversely, the reductions in protein degradation would have been greater but, as discussed below, also leading then to an apparently and unreasonably high net daily protein retention.

The 90-min periods of exercise increased leucine oxidation, which rapidly declined when the physical activity was terminated during the fasting state for both diets (Table 6). However, the exercise-induced increase in leucine oxidation appeared to be sustained into the early postexercise period when the activity occurred during the fed state, especially for the higher protein group, in which the oxidation rate postexercise was even higher than during exercise. The absolute increase in oxidation due to exercise was much greater for those receiving meals providing the 2.5-g level. For both fasting and fed states, whole body protein synthesis tended to decline during and/or immediately after exercise for both diet groups. Whole body protein degradation showed no changes during exercise for the fasting and fed states at both protein levels. However, for postexercise at both dietary levels, degradation was reduced during the fasting state and increased during the fed state (Table 6).

**DISCUSSION**

Our major purpose in bringing the present data from these two comparable metabolic studies together, with one having been published previously in part (13), was to examine the impact of the two different but adequate dietary protein intake levels on whole body leucine and urea N kinetics and leucine and N balances. This type of comparison has not been reported for a consecutive 24-h period previously. The consequences of the exercise periods on leucine kinetics and oxidation and on whole body protein turnover are of secondary importance for the present purpose, and the exercise-related

Table 5. Whole body leucine kinetics in adults given protein intakes as ten hourly small meals

<table>
<thead>
<tr>
<th>Condition</th>
<th>Leucine Flux</th>
<th>Leucine Oxidation</th>
<th>For Protein Synthesis</th>
<th>From Protein Degradation</th>
<th>Plasma Leucine, µmol/l</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fasting (10th hour)</td>
<td>137 ± 13a,f</td>
<td>36 ± 6a,f</td>
<td>101 ± 10b,g</td>
<td>129 ± 13a,f</td>
<td>105 ± 13</td>
</tr>
<tr>
<td>Fed (4th hour)</td>
<td>201 ± 17</td>
<td>92 ± 8</td>
<td>109 ± 19</td>
<td>19 ± 17</td>
<td>178 ± 24</td>
</tr>
<tr>
<td>25% Uptake*</td>
<td>249 ± 17a,f</td>
<td>114 ± 8a,f</td>
<td>135 ± 19a,f</td>
<td>50 ± 17a,f</td>
<td>178 ± 24</td>
</tr>
<tr>
<td>10% Uptake†</td>
<td>220 ± 17a,f</td>
<td>101 ± 8a,f</td>
<td>119 ± 19a,f</td>
<td>21 ± 17a,f</td>
<td>178 ± 24</td>
</tr>
<tr>
<td>Fasting (10th hour)</td>
<td>137 ± 13a</td>
<td>36 ± 6a</td>
<td>101 ± 10b,g</td>
<td>129 ± 13a,f</td>
<td>105 ± 13</td>
</tr>
<tr>
<td>Fed (4th hour)</td>
<td>201 ± 17</td>
<td>92 ± 8</td>
<td>109 ± 19</td>
<td>19 ± 17</td>
<td>178 ± 24</td>
</tr>
<tr>
<td>25% Uptake*</td>
<td>249 ± 17a,f</td>
<td>114 ± 8a,f</td>
<td>135 ± 19a,f</td>
<td>50 ± 17a,f</td>
<td>178 ± 24</td>
</tr>
<tr>
<td>10% Uptake†</td>
<td>220 ± 17a,f</td>
<td>101 ± 8a,f</td>
<td>119 ± 19a,f</td>
<td>21 ± 17a,f</td>
<td>178 ± 24</td>
</tr>
</tbody>
</table>

Values are means ± SD in µmol leucine·kg⁻¹·h⁻¹ unless otherwise stated. Experimental intervals: fasting, 90–150 min; fed, 540–600 min. *Corrected values, based on assumption of a 25% splanchnic first-pass removal of leucine and on 32 and 46% oxidation of this first-pass uptake at the 1- and 2.5-g levels, respectively (see METHODS). †Uncorrected values for fed state are not used in statistical analysis. Uptakes of 25 and 10% refer to assumed percentages for first-pass removal of absorbed leucine. †Corrected values, based on assumption of a 25% splanchnic first-pass removal of leucine. Between levels of protein intake (Student’s t-test); P < 0.01, †P = 0.06. Data for 1 g, 25% splanchnic first-pass removal vs. 2.5 g, 10% splanchnic first-pass removal (Student’s t-test): †P < 0.01, †P < 0.05. Plasma leucine: between levels (Student’s t-test), ‡P < 0.02; within 1-g level (paired t-test), †NS; and within 2.5-g level (paired t-test), ‡P < 0.001.
results for the 1-g level have been presented previously in detail (13). Therefore, these secondary aspects of whole body protein and amino acid metabolism, in relation to exercise, are covered here in brief by including new data for the 2.5-g level and comparing them in a summary form with data obtained at the 1-g protein intake level. A more extensive summary, however, of the data for macronutrient (carbohydrate, lipid, protein) oxidation, which can be derived from the calorimetric determinations made during the course of these studies, has yet to be presented.

Before we discuss the major findings, it is worth a moment to justify the approach used here to estimate whole body rates of protein synthesis and breakdown during the fed state. This metabolic condition introduces complexity in the interpretation of the plasma leucine kinetics and expired \(^{13}\text{CO}_2\) data when derived from the one-pool intravenous \(^{13}\text{C}\)leucine tracer model. Thus various approaches have been taken to examine whole body amino acid kinetics in the fed state, with some involving multitracer, intravenous, and oral routes of administration (6, 11, 26, 40) as well as determination of isotope levels in the free amino acid pool in plasma and liver-derived plasma proteins, especially apolipoprotein B-100 (3, 10, 38). However, all of these studies have limitations to the extent that no single model or approach can yet be taken as the “gold standard.” Therefore, although the approach used here may be regarded as being generally simplistic, it is based on our previous investigation (15) of this problem in which we assumed for subjects receiving a protein intake of 1 g·kg\(^{-1}\)·day\(^{-1}\) that 1) there was a 25% splanchnic first-pass disappearance (uptake) of total dietary leucine or a value consistent with our earlier direct studies (11, 26), and 2) the proportion of the dietary leucine entering this region that was oxidized was equivalent to the determined whole body oxidation, expressed as a percentage of the whole body leucine flux. These assumed values gave an estimate for daily endogenous leucine balance (protein synthesis – protein breakdown) that was very close to the measured daily balance (intake – oxidation) for subjects receiving an adequate leucine and protein intake, adding support for the assumptions made. Therefore, we considered it appropriate to apply this same approach here for estimating the changes in protein synthesis and breakdown with feeding at the present two dietary protein intakes.

Furthermore, a 25% splanchnic dietary uptake value is a reasonable approximation as found in our earlier studies (11, 26), although there are insufficient published data to propose a value that should be substantially different for a higher supramaintenance protein intake level. Hence we have assumed a 25% first-pass uptake of absorbed dietary leucine for both the 1 and 2.5 g·protein·kg\(^{-1}\)·day\(^{-1}\) intakes. Support for this comes from Cayol et al. (10), who estimated that splanchnic extraction was 30% with the use of a two-pool model and subjects given a protein intake of 1.5 g·protein·kg\(^{-1}\)·day\(^{-1}\). Because oxidation, relative to plasma flux, is dependent on the protein (leucine) intake we have computed here, the mean proportion of the splanchnic leucine uptake oxidized was 32% for the 1-g group and 46% for the 2.5-g level. In a previous study, the comparable value was 25%, as based on the flux and oxidation data (15).

It could also be questioned whether the assumed value of a 25% splanchnic extraction is reasonable, given the relatively variable tracer-derived estimates of the first-pass disappearance of absorbed leucine (9, 10, 11, 26, 40). Also, it might be that the percent extraction would decline with increased leucine intake, although this was not found to be the case in our previous study (11) involving a relatively low range of leucine intakes. Nevertheless, and although we give here (Table 5) estimates of the rates of protein synthesis and breakdown based on a 10% splanchnic extraction, we conclude that for the higher protein intake level, these rates would be too low, since the estimated difference between the protein balance during the

Table 6. Whole body leucine kinetics during and after exercise in adults given protein intakes for six days

<table>
<thead>
<tr>
<th>Metabolic State</th>
<th>Oxidation</th>
<th>For Protein Synthesis</th>
<th>From Protein Breakdown</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pre</td>
<td>During</td>
<td>Post</td>
</tr>
<tr>
<td>Fasting 1 g·kg(^{-1})·day(^{-1}) Protein intake (n=8)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fasting</td>
<td>28 ± 4(^a)</td>
<td>44 ± 7(^a)</td>
<td>27 ± 6(^a)</td>
</tr>
<tr>
<td>Fed</td>
<td>57 ± 6(^a)</td>
<td>(158 ± 23(^a))</td>
<td>(98 ± 15)</td>
</tr>
<tr>
<td>2.5 g·kg(^{-1})·day(^{-1}) Protein intake (n=6)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fasting</td>
<td>36 ± 6</td>
<td>52 ± 6</td>
<td>30 ± 3</td>
</tr>
<tr>
<td>Fed</td>
<td>114 ± 8(^c)</td>
<td>163 ± 24(^c)</td>
<td>199 ± 18(^c)</td>
</tr>
</tbody>
</table>

Values are means ± SD in μmol leucine·kg\(^{-1}\)·h\(^{-1}\) based on a 25% splanchnic uptake correction for the fed state as described in METHODS. Values in parentheses are percentages of preexercise rates. Significance between levels (1 vs. 2.5 g) in fasted state: \(^a\)P ≤ 0.05. Significance between levels (1 vs. 2.5 g) in fed state: \(^b\)P ≤ 0.05, \(^c\)P ≤ 0.01, and \(^d\)P ≤ 0.05. Fasted vs. fed state (within each protein intake level; paired t-test): \(^e\)NS, \(^f\)P < 0.01, and \(^g\)P ≤ 0.05. Within each dietary protein level, metabolic (fasted or fed) and variable (oxidation, synthesis, or breakdown) comparison among preexercise vs. during vs. postexercise (repeated-measures ANOVA followed by pairwise comparison among means using Student-Newman-Keuls test): \(^h\)different (P < 0.05) vs. other 2 situations; \(^i\)both equally different from preexercise situation; \(^j\)all 3 (pre, during, post) different from each other; \(^k\)different from preexercise only.
fasting and fed states would lead to a predicted daily protein balance that would be unreasonably positive. Indeed a higher percentage of extraction might well be operational at the 2.5-g intake on the basis of this line of reasoning, in which case the estimates of fed-state protein synthesis would be higher than we have derived. In summary, we conclude that the 25% assumption is reasonable for both protein levels, although it could possibly be particularly conservative for the 2.5-g level.

The two levels of protein intake studied in this investigation were chosen to exceed intakes considered to be sufficient to cover the dietary protein needs of essentially all healthy adults. Thus the 2.5-g level represents about a threefold higher intake than the recommended dietary allowance of 0.8 g protein·kg⁻¹ day⁻¹, as proposed by the United States Food and Nutrition Board (18). Therefore, the metabolic comparisons discussed below relate to an evaluation of the changes in protein, amino acid, and N kinetics that occur within the supramaintenance range of protein intakes.

With respect to the rate of urea production, we observed a quantitative difference between the normal and high protein intakes that is close to the absolute difference in total N intake. The absolute rate of hydrolysis of urea within the intestinal tract was the same for both levels of protein intake, with the percentage of total urea production undergoing hydrolysis being greater at the normal vs. high intake. This percentage of change with different intakes is consistent with previous studies (15, 28–30). Our finding that urea production rates in the two groups paralleled the difference in protein intake is not in accordance with the proposal put forth by Jackson (28) that there is no demonstrable association in adults between urea production and dietary intake over an adequate range of intakes, in excess of ~35 g protein daily. However, this view is based on limited data for higher protein intake levels. We conclude from the present data that over the surfeit range studied, the urea production rate responds in an essentially quantitative way to changes in the level of protein intake. The importance of this finding lies in the understanding of the role played by urea metabolism in the regulation of body N homeostasis, particularly because the "salvage" of urea N has been considered important in determining N balance under normal circumstances (30). Of course, it is possible that the physiological and metabolic significance of the relationships between N intake and urea kinetics differs in a complex way over a wide range of intakes, from inadequate or low intakes to those at around and above the upper level as we have examined here. This deserves further study, possibly using a 24-h tracer protocol as in the present investigation.

The physiological mechanisms responsible for the maintenance of body protein and amino acid homeostasis, particularly in relation to the impact of meals and the diurnal changes in protein turnover in human subjects, have attracted considerable attention in the past few years (6, 7, 10, 27, 35, 40). The reported effects of protein-containing meals on whole body protein synthesis have been quite variable, whereas whole body protein breakdown has often been estimated to be decreased (32). Additionally, the studies of Millward and co-workers (34, 35) have highlighted the importance of the level of protein intake on the diurnal nature of body N homeostasis, and they concluded that the amplitude of the diurnal cycling of body protein N metabolism increases with protein intake. In one of their studies (37), the test protein intake levels were 1.6 and 2 g protein·kg⁻¹ day⁻¹.

As presented above, with the use of the kinetic data that took into account the splanchnic metabolism of leucine, feeding increased protein synthesis by a mean of 10% in the normal protein group and 34% in the high protein group. Protein degradation decreased by ~30% at the 1-g protein level and 60% in the high protein group. Although the quantitative reliability of these changes depends on the assumptions made and the precise values used here to correct for the first-pass splanchnic metabolism of dietary leucine, there appear to be greater changes in protein synthesis and breakdown with ingestion of meals providing a greater surfeit of protein. The fact that whole body protein synthesis is estimated to be increased when an account of the splanchnic extraction is made is also consistent with the findings that there are increased rates of leucine incorporation into muscle (2, 22) and splanchnic (22) proteins as well as into whole body (22, 24) proteins when the feeding of amino acids is given by vein. In this latter case, tracer-model complications due to first-pass metabolism of the dietary tracee are obviated. Furthermore, our findings might be compared with those of Pacy et al. (37), who did not take into account the splanchnic metabolism of leucine in the evaluation of their [¹³C]leucine infusion model. Thus they showed that the higher fed-state protein gain between protein intakes of 1.6 and 2.0 g·kg⁻¹ day⁻¹ was essentially due to a greater inhibition of degradation, with no significant diet-dependent difference in the responses of protein synthesis to feeding, which increased by ~20% in both groups.

In another study, Gibson et al. (23) reported that, with ingestion of high protein meals (supplying the equivalent to ~1.5 g protein·kg⁻¹·day⁻¹), which followed 3 h of feeding with low protein meals (~0.2 g protein·kg⁻¹·day⁻¹), protein synthesis was stimulated to above the postabsorptive value by 11%. Although this is similar to the 10% increase found in our experiment with feeding at the 1-g level, it is difficult to compare closely these various findings because of the uncertainty of the conditioning, not only because the splanchnic uptake of dietary leucine was not taken into account but also because of the effect of the prior six small, low protein meals given at 30-min intervals in the study of Gibson et al. on the response to the following high protein meals.

Using their two-pool model, Cayol et al. (10) compared, for the fed state, the effects of feeding small complete meals that were protein free or contained protein to supply the equivalent of 1.5 g protein·kg⁻¹·day⁻¹. In
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