Effect of protein intake and physical activity on 24-h pattern and rate of macronutrient utilization

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Forslund, Anders H., Antoine E. El-Khoury, Roger M. Olsson, Anders M. Sjödin, Leif Hambraeus, and Vernon R. Young. Effect of protein intake and physical activity on 24-h pattern and rate of macronutrient utilization. Am. J. Physiol. 276 (Endocrinol. Metab. 39): E964–E976, 1999.—Effects of moderate physical activity (90 min at 45–50% of maximal O2 uptake 2 times daily) and “high” (2.5 g protein·kg−1·day−1, n = 6) or “normal” protein intake (1.0 g protein·kg−1·day−1, n = 8) on the pattern and rate of 24-h macronutrient utilization in healthy adult men were compared after a diet-exercise-adjustment period of 6 days. Energy turnover (ET) was determined by indirect and direct (suit) calorimetry, and “protein oxidation” was determined by a 24-h continuous intravenous infusion of [1-13C]leucine. Subjects were in slight positive energy balance during both studies. Protein contributed to a higher (22 vs. 10%) and carbohydrate (CHO) a lower (33 vs. 58%) proportion of total 24-h ET on the high- vs. normal-protein intake. The highest contribution of fat to ET was seen postexercise during fasting (73 and 61% of ET for high and normal, respectively). With the high-protein diet the subjects were in a positive protein (P < 0.001) and CHO balance (P < 0.05) and a negative fat balance (P < 0.05). The increased ET postexercise was not explained by increased rates of urea production and/or protein synthesis.

MANY RESEARCH STUDIES have been concerned with metabolic aspects of the relationship between physical activity, dietary intake, and body composition. However, a majority of these have focused on the short term (=9-h) effects of dietary intake and physical exercise on energy substrate metabolism. Although a number of 24-h studies have been performed to evaluate macronutrient utilization and its response to different dietary intakes (58), these have emphasized “total” 24-h macronutrient utilization and not the “pattern and rate” of macronutrient utilization throughout the 24-h period. Furthermore, the effect of a high-protein intake (vs. a normal-protein intake) on macronutrient metabolism has not been investigated in these previous studies.

Relatively high intakes of protein are often considered by athletes to be of benefit for vigorous exercise. Although convincing evidence seems to be lacking, it is known that high-protein diets alter hormonal status [e.g., changes in glucagon plasma levels (38)] and affect amino acid concentrations in circulating blood plasma. Furthermore, protein ingestion increases the rate of gluconeogenesis during the prandial phase of metabolism (31), and hence it can be hypothesized that these various responses to altered protein intakes also might serve to modulate the pattern and rate of macronutrient metabolism in response to exercise.

During “exercise,” carbohydrate (CHO) oxidation increases rapidly and may account for a dominant part of energy expenditure (12). As the exercise continues over time, fat oxidation increases (46), but the changes in amino acid oxidation are less clear cut. It has also been shown that CHO intake enhances body nitrogen retention to a greater extent than an equivalent intake of energy as fat (38). This is presumed to be related to the insulinogenic effect of CHO and its anabolic effect on protein metabolism (62). Consequently, when a mixed diet containing CHO is fed, it would be expected to spare amino acid oxidation. It could also be hypothesized that protein oxidation during exercise would be higher when it is performed in the fed state, as short-term changes in the catabolism of amino acids are dependent generally on their prevailing supply (i.e., substrate availability). During the “postexercise” period, there is an increase in energy turnover, and up to 50% of the increase has been attributed to increased triglyceride (TG)-fatty acid (FA) recycling (2).

These three different events (exercise, postexercise, and feeding) have been studied thoroughly via short-term studies and with a frequent focus on CHO and fat turnover. However, dietary protein and its role in energy consumption [ATP cost for protein synthesis (PS) and urea production] or energy transfer (ATP contributor through amino acid oxidation) during these different phases of increased energy turnover have received rather less attention.

In a previous study, we demonstrated, using a combined stable isotope labeled leucine/urea tracer protocol, that a daily moderate exercise program, while subjects were in approximate neutral energy balance and received 1 g protein·kg−1·day−1, did not measurably affect total 24-h protein oxidation (balance; see Ref. 15). We (23) also examined the effect of a high-protein intake (2.5 g protein·kg−1·day−1) on 24-h leucine and urea kinetics; however, we have not investigated the temporal changes in the “rates” of “oxidation” of protein, fat, and CHO in detail and their relative contributions to total energy turnover or how the dietary protein intake level modulates these rates.
Subjects and Methods

Eight healthy male volunteers participated in the normal-protein study, and six healthy male volunteers participated in the high-protein study. One of these individuals participated in both studies, and the results were reflective of the group mean differences. Descriptive data for the subjects are shown in Table 1. The subjects were recruited from the population of students and employees at Uppsala University. They were physically fit but not competitive athletes. All were in good health, as determined by medical history and physical examination; none of the subjects smoked or had excessive alcohol consumption. All subjects gave their written informed consent, and the study was approved by the Ethical Committee of the Faculty of Medicine at Uppsala University.

Experimental Design

Subjects were studied on an outpatient basis during days 1–5 at the nutrition metabolic unit. They ate the standardized diet for 7 days, and physical exercise was performed on a cycle ergometer within the unit. Day 6 was used as a so-called “sham infusion” day, and air samples were taken one time every hour to determine the change in background output of $^{13}$CO$_2$ in expired air (15, 16). O$_2$ uptake and CO$_2$ output were recorded at regular intervals throughout the day. In the evening of day 6, the subjects were dressed in the calorimeter suit, and intravenous catheters were inserted in the veins on the dorsal side of both hands, as previously described (15). Physiological saline was slowly infused during the night until the start of the tracer infusion at 0600 on day 7.

Day 7 was the L-[1-13C]leucine, [15N,15N]urea tracer infusion day, and the primed, continuous 24-h intravenous infusion of the stable isotope tracers was started at 0600 and continued until 0600 on day 8. The cycling exercise was performed between 0830–1000 and 1600–1730. When not cycling, the subjects usually sat in a chair while watching a video or reading.

Blood and expired air samples were collected every 30 min, except during exercise when they were collected every 15 min. During the night (2100–0600) breath samples were collected one time every hour. Urine samples were collected as complete, consecutive 3-h portions during the 24-h tracer infusion study on day 7. The experimental protocol for day 7 is depicted in Fig. 1; the experimental design and tracer protocol have been presented in detail in an earlier paper (15) and so this detail will not be repeated here. Estimates of whole body PS and of urea production were made precisely as previously described (15).

Body Composition

Subjects were weighed on a high-precision scale (Mettler, type KC120-ID1 Multirange; Mettler Instrumente, Greifensee, Switzerland) after 8 h of fasting on each morning of the study. A constant body weight was accepted as an indicator that the subject was in approximate energy balance. Body composition was determined just before the study began, using the three-compartment equation described by Forslund et al. (24).

![Fig. 1. Schematic outline of the experimental conditions during the 24-h tracer infusion study with indications for frequency of blood, breath, and urine samples as well as feeding and exercise periods. Continuous intravenous infusions of L-[1-13C]leucine and [15N,15N]urea, as well as direct calorimetry and indirect calorimetry, were performed throughout the 24-h period.](image-url)
Underwater weighing was used to measure body volume, and lung volume was measured by the helium dilution technique (Volugraph 2000; Mijnhardt, Bunnik, The Netherlands). Bioimpedance spectroscopy (XITRON 4000B; Xitron Technical, San Diego, CA) was used to estimate total body water.

Physical Capacity and Workload

Maximal O₂ uptake (VO₂max) was evaluated by an incremental test on a bicycle ergometer (Monark 829E; Monark Bodyguard, Vansbro, Sweden). O₂ consumption and CO₂ production were measured by an ergospirometer (model 2900Z; Sensormedics, Anaheim, CA) at 20-s intervals during the test, as the workload increased until exhaustion. The point at which ventilation and workload, but not O₂ uptake, increased was considered to represent VO₂max. The VO₂max was determined within 1 wk, or less, before the experiment began. The experimental physical activity program used during the study was set at 45–50% of VO₂max, which corresponded to an external workload around 100–110 watt.

The rate of O₂ consumption and CO₂ production during the exercise regime was also carried out during the 6 days before the tracer study on day 7. Food intake was monitored by recording daily dietary intake in both studies. The energy compositions of the diet during days 1–6 and during day 7 are shown in Table 2. 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.

Macronutrient and EnergyTurnover

The rate of O₂ consumption and CO₂ production during the 24-h study was assessed using a modified ergospirometer (Sensormedics 2900Z) that allows use of an open hood technique for up to a workload of 200 W, using an air flow rate of 270 l/min and permitting an O₂ uptake of 30 l/min. Autocalibration was performed every 30 min using two standard gases with known content of O₂ and CO2 (16% O₂ and 4% CO2, and 26% O₂ and 0% CO2 in nitrogen, respectively). Inspired air was checked every 10 min. Interpolations of O₂ and CO₂ were done during short periods (15 min) while the subject was eating and at every 8 h when a manual recalibration of the instrument was performed.

Irreversible protein nitrogen loss (or protein oxidation) was calculated as follows.

The nutrient content of the diet was estimated using software (MATs version 3.0; Rudans Data, Västerås, Sweden) based on the Swedish nutrient database from the National Food Administration (PC-diet, version 2–93). To calculate energy intake, body weight after an overnight fast was measured ~1 wk or less before the experiment. Basal metabolic rate was calculated from the FAO/WHO/UNU equations (18). For total energy expenditure, not including the experimental program of physical exercise, a physical activity level factor 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; next, the extra energy spent for physical exercise was added. The individual's diet was designed to keep the subject in energy balance and to supply either 1 g protein·kg⁻¹·day⁻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. Specially prepared cookies were used as an additional energy source, to balance energy expenditure. They were baked with a protein-free mix (low-protein and milk-free mix; Semper, Stockholm, Sweden), beet sugar, margarine, and sunflower oil and were flavored with raisins or chocolate. The cookies contained 0.3% energy from protein, 46% energy from fat, and 53.7% energy from CHO. The fat-to-CHO energy ratio in the nonprotein energy was 40:60 in both studies. The energy compositions of the diet during days 1–6 and during day 7 are shown in Table 2. 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.

### Table 2. Composition of the daily dietary intake during the 6-day adjustment period and on the 7th day when the 24-h tracer infusion study was done

<table>
<thead>
<tr>
<th></th>
<th>Normal Protein Intake</th>
<th></th>
<th>High Protein Intake</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>g·kg⁻¹·day⁻¹</td>
<td>MJ·day</td>
<td>E%</td>
<td>g·kg⁻¹·day⁻¹</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td><strong>Days 1–6</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Protein</td>
<td>1.0±0.0</td>
<td>1.44±0.11</td>
<td>8.0±0.4</td>
<td>2.5±0.0*</td>
</tr>
<tr>
<td>Fat</td>
<td>2.3±0.1</td>
<td>7.03±0.44</td>
<td>39.0±0.3</td>
<td>1.9±0.2*</td>
</tr>
<tr>
<td>Carbohydrate</td>
<td>7.8±0.5</td>
<td>9.56±0.60</td>
<td>53.0±0.2</td>
<td>6.5±0.7*</td>
</tr>
<tr>
<td>Total</td>
<td>18.02±1.12</td>
<td></td>
<td></td>
<td>17.64±1.11</td>
</tr>
<tr>
<td><strong>Day 7</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Protein</td>
<td>1.0±0.0</td>
<td>1.47±0.15</td>
<td>9.2±0.6</td>
<td>2.5±0.0*</td>
</tr>
<tr>
<td>Fat</td>
<td>2.0±0.1</td>
<td>6.19±0.42</td>
<td>38.8±0.4</td>
<td>1.6±0.2*</td>
</tr>
<tr>
<td>Carbohydrate</td>
<td>6.8±0.5</td>
<td>8.31±0.61</td>
<td>52.0±0.3</td>
<td>5.5±0.6*</td>
</tr>
<tr>
<td>Total</td>
<td>15.98±1.09</td>
<td></td>
<td></td>
<td>15.54±0.94</td>
</tr>
</tbody>
</table>

Data are means ± SD. E% percent of total energy intake. *Significantly different from normal protein intake (P < 0.001; t-test for independent samples). †Significantly different from normal protein intake (P < 0.01; Mann-Whitney U-test).
where

\[ ^{13}\text{CO}_2 \text{ production (µmol·kg}^{-1} \cdot 30 \text{ min}^{-1}) = V^{13}\text{CO}_2 (µmol·kg}^{-1} \cdot 30 \text{ min}^{-1}) \times ^{13}\text{CO}_2 \text{ enrichment (APE } \times 1,000) \times \frac{1}{1.05} \times \frac{1}{R} \]

where APE is atom percent excess; \(^{13}\text{CO}_2\) enrichment is the average of the two enrichments at the two time points determining the half-hour interval (this average was corrected for \(^{13}\text{CO}_2\) background, as described earlier; Ref. 15); and 1/R is the [\(^{13}\text{C}\)]bicarbonate fraction recovered during each 30-min interval (or 15-min interval for the exercise period), as previously described (15). [\(^{13}\text{C}\)]KIC enrichment is the average of the enrichments at the beginning and end of each half-hour interval (or 15-min interval during exercise).

Irreversible protein nitrogen loss. From the estimates of leucine oxidation, irreversible protein nitrogen loss (mg protein·kg\(^{-1}\)·day\(^{-1}\)) was calculated as:

\[ \text{[24-h leucine oxidation (mg·kg}^{-1} \cdot \text{day}^{-1}) = 24\text{-h leucine tracer given (mg·kg}^{-1} \cdot \text{day}^{-1}) \times \left( \frac{100}{8} \right) \times \left( \frac{8}{10.03} \right) \] (15).

Rates of whole body PS and urea production were calculated as described in detail previously (23). Calculation of the ATP cost for PS and urea production was made assuming that 6 mol ATP are used per 110 g PS and 4 mol ATP/mol urea produced. We further assumed that, to replace 1 mol ATP, this required an average of 24 kcal. These assumptions are presented in detail elsewhere (63).

The net macronutrient utilization of fat and CHO was calculated according to Garlick's (25) equations. To determine the contribution made by the macronutrients to energy expenditure the following constants were used: protein 18.42 kJ/g, fat 39.14 kJ/g, and CHO 15.70 kJ/g (25). Energy turnover was calculated by adding the energy values from the macronutrients together. The rate of macronutrient turnover was calculated for each consecutive 30-min period throughout the 24-h infusion day. Rates were then expressed as milligrams or joules per kilogram body weight per unit time. Twenty-four-hour direct calorimetry was continuously performed using the suit calorimeter, as previously described (27).

To estimate the nonprotein respiratory quotient (RQ), the amount of O\(_2\) consumed and CO\(_2\) produced during the oxidation of the dietary milk protein was calculated (14). Oxidation of 1 g milk protein was estimated to consume 1.025 l of O\(_2\) and produce 0.841 l of CO\(_2\) (milk protein RQ = 0.82), and this was subtracted from the total amount of O\(_2\) used and CO\(_2\) produced. Also, calculation of nonprotein food quotient (FQ) was performed (5). Because milk protein was used in the diet, milk nonprotein RQ will be given in the results. A nonprotein RQ using beef as the protein source was also calculated. However, this did not significantly alter the results, so these data will not be shown.

In addition to the continuous 24-h pattern and rate of macronutrient utilization, the 24-h period was divided into seven separate 90-min periods representative of different metabolic/physiological conditions as follows: for “fasting” the 0600 to 1200 period was divided into preexercise (0700–0830), exercise (0830–1000), and postexercise (1000–1130). During “feeding” (1200–2100) the periods were as follows: preexercise (1430–1600), exercise (1600–1730), and postexercise (1730–1900). The fasting state during the night was taken to be from 0430 to 0600.

Statistics

The software program STATISTICA version 4.5 (StatSoft, Tulsa, OK) was used for all statistical calculations. A simple regression analysis was used to calculate correlations. Wilcoxon’s match pair test was used to evaluate the possible differences when energy percentage values were compared within each study. Mann-Whitney U-test was used to evaluate the possible differences when energy percentage values were compared between the two studies. To calculate significant differences between rates expressed as milligrams or joules per kilogram per time unit, a t-test for dependent samples was used within each study and a t-test for independent samples was used between the two studies. All values refer to day 7 and are expressed as means ± SD, unless otherwise indicated.

RESULTS

The subjects maintained a stable body weight during the 7-day study. Although there was no significant difference between the indirect and direct calorimetric estimations of total energy turnover, indirect calorimetry indicated a slight but significant (P < 0.05) positive energy balance (equivalent to 5% of intake) during the 7th day (the infusion day) during the normal-protein study. Balance was not significantly different from equilibrium when estimated via the direct calorimetric method (Table 3).

The pattern and rate of “protein” utilization is shown in Fig. 2. During exercise, while subjects were in the fasting state, protein utilization increased to the same degree, although the absolute rate was significantly higher during the high-protein study. After termination of exercise (at 1000), protein oxidation decreased, reaching significantly lower values at 1230 compared with the preexercise value at 0830 in both groups (P < 0.05; Fig. 2).

Feeding increased protein oxidation, and the increase was more pronounced for the high-protein group. During exercise, protein utilization increased in both groups, and termination of exercise was associated with an immediate and significant (P < 0.01) decline in protein oxidation. It later increased for a 60-min period, rising to the same level as during early exercise in the normal-protein group, whereas with the high-protein group it showed a tendency to reach above the highest oxidation rate obtained during exercise. After the end of the feeding period, protein oxidation declined progressively in both groups (Fig. 2).

When calculating the extra protein oxidized during exercise, using as a baseline the value obtained just before exercise, there was a significantly (P < 0.05) higher protein utilization during the exercise period in feeding (66.3 ± 37.4 and 35.4 ± 8.4 mg with the high and normal-protein diets, respectively) compared with the change in fasting (28.1 ± 12.7 and 24.4 ± 6.9 mg). When comparing the diets, the high-protein group had
a higher protein utilization during feeding (P = 0.04) but not during fasting.

The 24-h pattern and rate of “fat” utilization is shown in Fig. 3. During exercise, while subjects were fasting, fat utilization increased. Postexercise fat utilization slowly decreased, and it continued to decrease during feeding (P < 0.01), becoming lower than the preexercise value by 1530. The effect of physical exercise on the fat utilization rate was lower (P < 0.01) in the fed vs. fast state in both groups. As shown in Fig. 3, there was a higher fat utilization rate in the high-protein group despite a lower fat intake.

The pattern and rate of CHO utilization is shown in Fig. 4. Exercise during fasting promptly increased CHO utilization, and during postexercise CHO utilization decreased markedly, falling below the preexercise fasting value in both groups. Feeding increased CHO utilization and to a higher degree when the normal-protein diet was given. Exercise during feeding stimulated CHO oxidation and to a higher degree than during fasting for subjects given the normal-protein intake.

During the overnight fasting period, protein oxidation (P < 0.01) slowly and progressively decreased in both groups. Also, CHO (P < 0.01) utilization slowly and progressively decreased, whereas fat utilization slowly increased (P < 0.05) only in the normal-protein group, compared with values recorded at the end of the feeding period.

The nonprotein RQ (FQ) for each group throughout the 24-h study period is shown in Fig. 5. The nonprotein FQ was the same in both groups (0.87); it was significantly different (P < 0.01) from the nonprotein RQ in Table 3.

Table 3. Estimates of energy turnover and balance during day 7 of the 2 different experiments

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Normal Protein Intake</th>
<th>High Protein Intake</th>
</tr>
</thead>
<tbody>
<tr>
<td>Energy turnover</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Indirect calorimetry</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Protein</td>
<td>1.53 ± 0.2</td>
<td>3.31 ± 0.57*</td>
</tr>
<tr>
<td>Fat</td>
<td>4.95 ± 0.2</td>
<td>6.78 ± 2.35</td>
</tr>
<tr>
<td>Carbohydrate</td>
<td>8.63 ± 0.2</td>
<td>4.82 ± 1.02*</td>
</tr>
<tr>
<td>Total</td>
<td>15.12 ± 1.2</td>
<td>14.81 ± 1.85</td>
</tr>
<tr>
<td>Direct calorimetry</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Evaporation</td>
<td>3.76 ± 0.67</td>
<td>2.94 ± 1.80</td>
</tr>
<tr>
<td>Convection + radiation</td>
<td>10.36 ± 0.74</td>
<td>10.57 ± 0.73</td>
</tr>
<tr>
<td>External workload</td>
<td>1.18 ± 0.15</td>
<td>1.06 ± 0.04</td>
</tr>
<tr>
<td>Total</td>
<td>15.30 ± 1.21</td>
<td>14.57 ± 2.34</td>
</tr>
<tr>
<td>Energy balance</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Indirect calorimetry</td>
<td>0.86 ± 0.84†</td>
<td>0.73 ± 1.03</td>
</tr>
<tr>
<td>Direct calorimetry</td>
<td>0.68 ± 0.87</td>
<td>0.97 ± 1.70</td>
</tr>
</tbody>
</table>

Data are means ± SD. *Significantly different from normal protein intake (P < 0.001; t-test for independent samples). †Significantly different from zero balance (t-test for independent samples). ‡Significantly different from normal protein intake (P < 0.01; Mann-Whitney U-test).
the high-protein group (0.82) but not for the normal-protein group (0.88). The 24-h nonprotein RQ for the normal-protein group was also significantly (P < 0.02) higher than that for the high-protein group.

Twenty-four hour macronutrient balances are shown in Fig. 6. Although for the normal-protein group there was a tendency for a small mean negative protein and CHO balance and an equally small mean positive fat balance, none of these were significantly different from body equilibrium or zero balance. However, in the high-protein group there was a slight positive protein balance, positive CHO balance, and a tendency for a small negative 24-h fat balance [not significant (NS)]. However, when fat balances between the two dietary protein levels were compared, the fat balance was significantly lower during the high- vs. normal-protein intakes.

The absolute contribution of the different macronutrients to the rate of energy turnover is illustrated in Fig. 7, A and B. Figure 7 shows the dominance of CHO as an energy source during exercise, especially with the normal-protein intake, and shows that this dominance was less pronounced during the fasting compared with the fed state. An increase in absolute values for protein oxidation was observed during exercise in both groups; however, regarding the relative contribution of macronutrients to energy turnover, it is evident that there is a decrease in the proportionate contribution from protein to energy turnover during exercise in both groups (Fig. 8, A and B).

Figure 8 summarizes the mean relative contribution of the macronutrients to energy turnover during selected periods of the 24-h protocol, with comparisons made between the two studies. The high-protein group showed a higher protein and fat contribution and a lower CHO contribution throughout the 24-h study, during the different periods, compared with the normal-protein group. "Physical exercise" decreased the contribution (%) from protein and increased the contribution from CHO to energy flux in fasting and during feeding in both dietary groups. Fat accounted for 38% in the normal-protein group and 48% in the high-protein group of energy expenditure during exercise in fasting, which was the same as for resting energy expenditure, and for a lower level, 14 and 28%, respectively, during the fed state. Fat is the major energy source postexer-
Exercise during fasting, being up to 71% in the high-protein study and up to 60% in the normal-protein study. In feeding, protein contribution to energy turnover was higher during the high-protein study, and in postexercise (in the fed state) up to 61% of the energy came from protein compared with 24% with the normal-protein intake.

Table 4 presents mean values for total energy turnover during selected periods and again with a comparison between the two studies. Table 4 also shows the fraction (as %) of the energy turnover related to PS and urea production. There was no difference in total energy turnover between the two diet groups at the specific different periods, although with the normal-protein intake there was a tendency for a higher energy turnover during exercise compared with the high-protein intake. The proportion of the whole body energy turnover due to PS and urea production decreased during exercise in both groups (Table 4).

Feeding increased energy turnover by 22–24% from the preexercise fasting phase to the preexercise feeding phase (P < 0.01) in both groups. This increase corresponded to 5–6% of energy intake. The energy cost for PS increased from 15.8 ± 1.7 during resting metabolic rate (RMR) conditions to 18.2 ± 1.5 J·kg⁻¹·min⁻¹ during the fed period and from 14.4 ± 1.4 to 20.2 ± 2.8 J·kg⁻¹·min⁻¹ in the normal- and high-protein groups, respectively. This increase corresponded to 16 and 42% of the thermic effect of food (TEF) in the normal and high-protein diets, respectively. The energy cost for urea production in absolute terms and its relative proportion of energy turnover were significantly higher (Table 4) in the high-protein group compared with the normal-protein group both during the fast and fed periods. There was no contribution from urea production to TEF. However, the change in the proportion of the energy turnover that went into PS and urea production due to feeding was not significantly different (Table 4).

There was a significantly higher energy turnover during the 90-min postexercise period compared with the 90-min period before exercise during fasting and during feeding. The differences were of the same magnitude for the fasting and fed states (12–15%) in the normal-protein group. However, in the high-protein group, it was a 12% increase in the fasted state and a 26% increase in the fed state. This difference in increased energy turnover was not explained by a change in PS or urea production rate. PS showed a decrease postexercise.

DISCUSSION

Study Design Characteristics

The “aim” of this study was to evaluate the effect of a constant and well-defined dietary intake with either...
Table 4. Energy turnover at various times during 24-h studies, before, during, and after exercise, in fast and fed states at normal or high protein intake and percentage of energy turnover used for protein synthesis and urea production, respectively.

<table>
<thead>
<tr>
<th>Period (real time)</th>
<th>Normal (n = 8)</th>
<th>High (n = 6)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Energy turnover, J·kg⁻¹·min⁻¹</td>
<td>Urea production, %</td>
</tr>
<tr>
<td></td>
<td>Fasting</td>
<td>Feeding</td>
</tr>
<tr>
<td>Preexercise (0700–830)</td>
<td>79 ± 9.5a</td>
<td>20.1 ± 2.7a</td>
</tr>
<tr>
<td>Exercise (0830–1000)</td>
<td>482.4 ± 44.7a</td>
<td>2.7 ± 0.3b</td>
</tr>
<tr>
<td>Postexercise (1000–1130)</td>
<td>91.8 ± 13.3c</td>
<td>16.1 ± 2.2c</td>
</tr>
<tr>
<td>Preexercise (1430–1600)</td>
<td>98.4 ± 10.2c</td>
<td>18.6 ± 1.3a</td>
</tr>
<tr>
<td>Exercise (1600–1730)</td>
<td>497.6 ± 49.4a</td>
<td>3.2 ± 0.7d</td>
</tr>
<tr>
<td>Postexercise (1730–1900)</td>
<td>110.4 ± 10.1c</td>
<td>15.4 ± 2.3c,e</td>
</tr>
<tr>
<td>Night (0430–0600)</td>
<td>69.9 ± 5.8b</td>
<td>18.8 ± 2.5a</td>
</tr>
</tbody>
</table>

Data are means ± SD; n, no. of subjects. *Significantly different from normal protein intake (P < 0.05). Independent t-test was used when evaluating differences in energy turnover, Mann-Whitney U-test was used when evaluating differences in energy cost for protein synthesis (PS) and urea cycle. Means in the same column that have different superscript letters are significantly different (P < 0.05). Dependent t-test was used when testing differences between energy turnover; Wilcoxon’s match pair test was used when evaluating differences in the energy cost for PS and urea cycling within each group.

The “dietary intake” was chosen to maintain body energy balance, with body weight stability taken as an indication of balance during days 1–6. The energy balances obtained during day 7 by direct and indirect calorimetry were not significantly different from each other and indicated that our subjects were at a neutral energy balance. However, during the high-protein diet, direct calorimetry revealed a higher 24-h energy expenditure than that obtained with indirect calorimetry compared with the normal-protein study where the opposite was found (NS).

Direct calorimetry measures total “heat loss” from the body; indirect calorimetry measures the oxidation of substrates as an indication of total “energy transfer and retention” body (19); therefore, it is possible that they could show different values, under certain circumstances, possibly related to uncoupling between oxidative metabolism and ATP function. Amino acids are the major fuel of liver, and the daily supply of amino acids provided in the diet cannot be totally oxidized to CO₂ in the liver because such a process would provide far more ATP than the liver could utilize (31). During amino acid oxidation the liver mitochondria experiences a severe nonvolatile acid load, and this load is alleviated mainly by the respiratory chain proton pump in a form of uncoupled respiration (31), which would probably be more pronounced at a high-protein intake. According to Rolf and Brown (45) the Na⁺, K⁺, H⁺, and Ca²⁺ channels and leaks of cell membranes and protein breakdown could cause uncoupling. Uncoupling would then produce less ATP and more heat per liter of O₂ consumed. Thus the uncoupling of respiration due to the high-protein intake might then provide an explanation to why indirect calorimetry showed a slightly higher energy turnover value than direct calorimetry during the normal-protein study and a lower value during the high-protein study.

The physical exercise comprised two separate 90-min periods, with a physical work intensity corresponding to 45–50% of VO₂max. This exercise program would have been difficult to sustain in normally fit healthy males during a 7-day period if the intensity of the exercise had been >50% VO₂max, a level used, for example, in the exercise study of Devlin et al. (13). Furthermore, most studies on the effect of physical exercise on protein turnover, using stable isotopes of leucine, have used a level of physical exercise of a comparable magnitude as our study (10, 60). Another reason to keep the exercise intensity at a low-to-moderate level is that it might be argued that a 1-wk period of 3 h of more vigorous exercise per day would have resulted in a training effect. For example, it has been shown that 10 days of 2 h of training per day at 60% VO₂max (36) reduce glucose production and utilization during exercise. In our study there was a tendency, which was not significant, for a decrease in the mean exercise RQ as the
week progressed. Thus, by maintaining the physical exercise load within a narrow and moderate range, in relation to the individual’s $V_{\text{O2max}}$, and also while keeping the subjects sedentary between the exercise periods, the physiological status of our subjects was considered to have been very well standardized.

Although this exercise regime corresponds to a basal metabolic rate factor of 6.1, it may be considered to represent a degree of physical exercise of low-to-moderate intensity, corresponding to a physical activity level factor of 1.7 (30). Thus with 3 h of physical exercise per day there was an extra energy turnover of $\sim 5.5 \text{ MJ/24 h or a 36\% increase in 24-h energy turnover due to bicycle exercise.}$

**Macronutrient Utilization During Basal or Resting Metabolic Conditions**

The contribution of macronutrients to energy turnover during basal metabolic rate conditions is dependent on duration of fasting (9), preceding diet (41), nutritional state (43), and training background (47). This makes it difficult to strictly compare the present data with that from many of the published studies by other investigators. Nevertheless, during the normal-protein intake, our determined rate and contribution of protein and CHO utilization to energy turnover agree well with that reported by various investigators (9, 17, 26), in spite of small differences in the duration of fasting before measurements were made in the different studies.

With respect to fat utilization, Garlick et al. (26) estimated a slightly higher rate compared with our normal-protein group ($1.18 \pm 0.15$), but their data were obtained after a 13- to 17-h fast. Rominj et al. (47) also showed a slightly higher rate of fat utilization compared with our values after a 12-h fast in endurance athletes ($1.46 \pm 0.11$) and in a nonathlete group ($1.06 \pm 0.11$). However, their subjects were fed a lower amount of CHO (4.2 g·kg$^{-1}$·day$^{-1}$) on the preceding day compared with our subjects (6.8 g·kg$^{-1}$·day$^{-1}$), which may be an explanation for the differences noted. When athletes were fed a higher amount of CHO (8.6 g·kg$^{-1}$·day$^{-1}$) on the preceding day, fat utilization was lower (47). Finally, our estimates of fat and CHO utilization rates agree well with those for the healthy controls in a study by Raguso et al. (42).

During the high-protein study, we found a higher protein and lower CHO contribution to energy turnover both in absolute (Fig. 7) and relative (Fig. 8) terms during RMR conditions and a higher relative contribution from fat. Unfortunately, there are no other comparative studies on the effect of a high-protein diet after a 6-day diet-exercise adjustment period on macronutrient utilization; high-protein intake studies appear to have largely explored the acute diet effect on TEF.

The RMR for our subjects are consistent with our earlier findings (53) ($81.9 \pm 14.4$) and those by Rominj et al. (77.9 ± 4.1; see Ref. 47) in endurance athletes. As the duration of fasting (9) and body composition (43) may influence RMR measurements, it should be noted that the subjects in the present study had a relatively higher amount of fat-free body mass than the nonathletes in our previous study (53). Furthermore, when values from different studies are compared, it must be noted that the RMR values derived are dependent on the particular equations used to account for protein (nitrogen) loss. Finally, we cannot rule out the fact that the present RMR values are influenced by the stress due to the experimental conditions, which included wearing the calorimeter suit and taking blood samples.

Rominj et al. (47) estimated the energy cost for TG-FA cycling to be 4.4 and 1.2% of RMR in endurance athletes and in a sedentary control group, respectively. Here, for comparison, we estimated that the energy cost of PS and urea production accounts for $\sim 18$–$20$% and 2–4% of RMR in the two diet groups. Also, it might be pointed out that the energy cost for PS was higher than the energy contribution from protein oxidation during RMR conditions.

**Effect of Exercise on Macronutrient Utilization**

The rate of macronutrient utilization during exercise is dependent on the length and both absolute and relative intensity of the workload (40, 46), the nutritional state (49) of the subjects, and the training background (29, 32). Again, this makes it difficult to strictly compare the present data with that from many of the published studies by other investigators. Although the rate of protein utilization was increased by exercise, we did not find any effect on the total 24-h protein utilization when expressed as gram protein per kilogram per day (15). This appears in contrast to suggestions that have been made by some investigators (34), although differences in experimental design may account for this. However, when protein utilization is related to total energy turnover, the contribution of protein as an energy source decreased during exercise compared with preexercise conditions. The fact that protein contributes only to a minor part of energy turnover during exercise has been addressed earlier by Calles-Escandon et al. (8); however, they used urea excretion as an index of protein degradation, which may not be as precise as the present $[13\text{C}]$leucine approach.

Exercise during fasting led to a higher fat utilization and lower CHO utilization when compared with exercise during feeding, irrespective of whether the data are expressed in absolute (as mg·kg$^{-1}$·min$^{-1}$; Fig. 7) or in relative (as % of energy expenditure; Fig. 8) terms.

The significant role of CHO as an energy source during exercise, especially during feeding, has been noted by many investigators (52). For our subjects in the normal-protein group, 7.0 g CHO/kg body weight were utilized during the 24-h study, which is close to the rate previously reported for people engaged in endurance exercise (12), and accounted for close to 100% of the CHO consumed. However, in the high-protein group only 3.9 g CHO/kg body weight were oxidized daily, amounting to $\sim 60$% of intake.

Coggan et al. (11) studied glucose kinetics, using glucose and bicarbonate labeled with stable isotopes, in subjects exercising at 60% of $V_{\text{O2max}}$. Their energy
turnover corresponded to 542 J·kg⁻¹·min⁻¹, with 37% of the energy derived from fat and 63% of the energy from CHO, close to the values reported here. Finally, Rominj et al. (47) determined the effect of exercise intensity and duration on substrate utilization in endurance-trained subjects using glucose, glycerol, and palmitate labeled with stable isotopes. At a workload of 25% VO₂max (~1.25 l/min) fat oxidation corresponded to 3.8 mg·kg⁻¹·min⁻¹ and CHO oxidation to 7.7 mg·kg⁻¹·min⁻¹. At a higher workload, 65% of VO₂max (~3.25 l/min), fat oxidation corresponded to 26.1 and CHO oxidation to 12.3 mg·kg⁻¹·min⁻¹. Taking into consideration that our subjects were physically fit, but not endurance trained, and that they exercised at a workload of 45% of VO₂max (~1.86 l/min), our reported rates of fat and CHO oxidation are entirely consistent with these earlier findings.

Effect of Feeding on Macronutrient Utilization

Protein utilization increased gradually during feeding in both groups, but this response was more pronounced in the high-protein group. In both groups feeding per se had a greater effect on protein utilization than did exercise. The average rate of protein utilization after feeding with a normal-protein diet agrees with earlier findings (16, 17), and, for conditions similar to those for the present experiment, oxidation reaches a steady state after 2–3 h (16, 17). However, it could be questioned if steady state was reached on the high-protein diet as there was a much higher increase in protein utilization postexercise vs. that for the normal-protein diet. Nevertheless, to study the interaction between feeding and exercise, we chose to start the exercise after 4 h of feeding.

In the normal-protein group we found that CHO intake promoted CHO utilization while fat intake did not promote fat utilization, which is consistent with the work of Flatt et al. (22) and Schutz et al. (51).

With respect to total energy turnover, feeding induces thermogenesis (TEF). Usually TEF is reported as the energy turnover during feeding divided by the RMR (57). Our values for TEF compare well with the published data. Both Tai et al. (55) and Thörne and Wahren (57) showed a postprandial increase in energy expenditure above basal values, of ~25%, corresponding to 6% of ingested energy. However, they did not measure protein utilization and assumed a constant protein oxidation rate during the study, which was not the case in the present study.

Protein has been considered as the macronutrient with the highest TEF. In our study, we found an increase in the energy cost for PS, which explained 16 and 42% of TEF in the normal and high-protein diet, respectively. However, we were not able to find a change in the proportion of the energy turnover due to the energy cost of PS and urea production when comparing the high- and normal-protein intake. Robinson et al. (44) found that PS accounted for 11.7% of total energy expenditure using a normal-protein diet (0.9 g protein·kg⁻¹·day⁻¹) and 19.8% when a very-high-protein diet (4.3 g protein·kg⁻¹·day⁻¹) was fed. They further calculated that 36% of TEF at the normal-protein diet and 68% of TEF at the high-protein diet were explained by an increased PS, i.e., higher vs. our study (16 vs. 42%). It should be noted that Robinson et al. (44) studied the acute effect of a high-protein intake using an oral dose of [¹⁴N]glucose. They also calculated the energy cost of urea synthesis and found that it was 9 and 28% of TEF in the normal- and high-protein intake groups, respectively. The combined energy cost of protein and urea synthesis would then contribute 45 and 96% of TEF in the normal- and high-protein groups, respectively.

Changes in Macronutrient Utilization Postexercise

The term excess postexercise O₂ consumption (EPOC) refers to the increased rate of “energy turnover” seen after exercise (3). The major extrinsic factors influencing EPOC are duration and intensity of exercise. Intensities below 70% of VO₂max do not appear to have a prolonged effect on EPOC unless the duration of exercise is long (41). Barshèm et al. (6) have shown that elevated norepinephrine levels may be important for EPOC, as they are elevated in the early phases of EPOC but return to normal within a few hours. Thus we chose the first 90-min postexercise period when evaluating the effect of fasting or feeding and a normal or high-protein diet on EPOC. A significant part of EPOC has been related to the increased TG-FA cycle activity (2, 61). The EPOC found in our study, both in fasting and feeding, at a normal-protein intake agrees with data of Bahr and Sejersted (3). When a high-protein diet is fed, EPOC showed an increase of 26%, and this was not explained by a change in PS and urea production.

During fasting, both protein and CHO utilization decreased rapidly with termination of exercise. The mean protein utilization values were not significantly lower than preexercise values, but the lowest postexercise values, obtained after 150 min, were significantly lower, irrespective of the preexercise values chosen as baseline. Devlin et al. (13) showed that leucine oxidation decreased postexercise when the subjects exercised at 75% of VO₂max until exhaustion. Our study shows a similar effect during a fast period in response to low-to-moderate physical exercise. From our previous paper in this series, it is clear that the increased leucine oxidation during exercise is compensated for within a 24-h time frame (15), at least when the intake of leucine exceeds estimated physiological requirements (18).

With respect to the fed period, however, there was no decrease in protein utilization postexercise, and at 60 min postexercise it actually started to increase, reaching a peak level after 120 min postexercise; in the high-protein diet group it even increased above exercise values. One possible explanation could be that postexercise there is an increased amino acid uptake in the cell (8) and possibly more so on a high-protein diet, leading to a rise in the oxidative fate of the amino acids.

Previous studies (3, 4) have shown a lower utilization rate of CHO postexercise, and this was confirmed in our study for fasting and feeding when expressed in rela-
Twenty-Four Hour Energy and Macronutrient Balance

One might argue that the higher fat and lower CHO utilization obtained on the high-protein diet compared with a normal-protein diet may be due to individual differences between the two study groups such as genetic differences, as suggested by studies in twins (7), muscle fiber type (33), distribution of lipoprotein lipase among muscle and adipose tissue (20), and/or physical fitness (47). This seems unlikely, and the one subject who participated in both studies had a higher fat oxidation and lower CHO oxidation on the high-protein diet, which argues against individual metabolic differences between the two study groups. Another possible explanation in theory could be a difference in energy balance between the two groups; however, this was not the case here.

A somewhat lower CHO intake for 7 days would possibly lead to a smaller glycogen store on day 7 and then perhaps a lower CHO oxidation, with a compensatory increase in fat oxidation. However, although the subjects in the high-protein group received less endogenous CHO (434 vs. 539 g in the high- and normal-protein groups, respectively), the calculated amount of endogenous CHO from gluconeogenesis on the high-protein diet would contribute to ~100 g of endogenous CHO. This would mean that our subjects received almost the same total (exogenous and endogenous) amount of CHO with both diets. The biochemical mechanism responsible for the apparent sparing of CHO and enhanced fat oxidation when the high-protein diet was given cannot be identified from the present data. However, we might speculate that the relative sparing of glucose and enhanced utilization of fat when the high-protein diet was consumed are the results of a higher circulating glucagon level and a lower plasma insulin-to-glucagon ratio (Forslund, Hambraeus, and Young, unpublished results). Glucagon stimulates lipolysis (48), mediates ketone body production, and raises free FA concentrations, which would be expected to favor FA catabolism and oxidation. Therefore, the relative shift toward lipid oxidation and glucose sparing may be due to the altered glucagon/insulin relationship brought about by the high-protein feeding. It would be difficult to test this hypothesis under chronic conditions of a high-protein intake. However, it could be tested after acute changes in the level of dietary protein, using a hormonal clamp technique as has been applied, for example, in studies of whole body amino acid kinetics (35).

Temporal Pattern and Rate of Macronutrient Utilization

The role of macronutrient depot sizes in macronutrient homeostasis has been addressed by various authors (21, 28, 37). Flatt (21) has raised the hypothesis that CHO utilization is more tightly regulated (vs. fat utilization) due to the limited storage capacity for CHO, whereas fat utilization is determined by the degree of expansion of the adipose tissue mass. Stubbs (54) has proposed a "hierarchy" in oxidation, where the priority in oxidation is highest for protein and lowest for fat. Our results appear to support these concepts.

CHO (1, 59) and protein intake or availability (16) stimulate their own oxidation; fat intake, on the other hand, has little impact on fat oxidation (22). However, when lean subjects were compared with obese subjects (50, 56), a mild relationship between fat intake and fat utilization was found, which could be explained by individual differences in fat pool sizes. Consequently, it seems that the availability of the different macronutrients in relation to their respective pool sizes has an influence on the 24-h temporal pattern and rate of macronutrient utilization. This may provide the means for whole body macronutrient homeostasis and could help explain the pattern found in our study. In reference to protein, it may also be postulated that the intracellular amino acid concentration, per se, has an impact on the utilization rate (oxidation).

The utilization of macronutrients from the intracellular storage sites is increased during exercise (46). When glycogen depots are mobilized this could lead to intracellular water loss, as glycogen "binds" three to four times its weight of water (39). Hence, a reduced cell volume might lead to an increased intracellular amino acid concentration, which secondarily leads to an increased protein utilization (oxidation) rate. During the postexercise period, there is a reduced CHO and protein utilization, more clearly in the fasted state, that also in part might be explained by substrate availability in relation to individual pool size. However, more studies are needed to further understand the mechanisms responsible for these findings.

We have evaluated the impact of dietary protein level on energy turnover and substrate utilization during a 24-h period including exercise under conditions of neutral energy balance. A high-protein intake induced more negative fat and more positive CHO balances compared with a normal-protein intake. This might be explained, at least in part, by the effect of an altered protein intake on the interrelationship between circulating insulin and glucagon levels. A question emerging from our findings is whether a high- compared with a normal-protein intake, which appears to spare CHO loss and therefore presumably attenuate glycogen depletion with vigorous prolonged exercise, would favor endurance during a sustained period of moderate-intensity exercise.

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