Protein turnover and thermogenesis in response to high-protein and high-carbohydrate feeding in men1-3

Sian M Robinson, Christian Jaccard, Chandarika Persaud, Alan A Jackson, Eric Jequier, and Yves Schutz

ABSTRACT The rates of energy expenditure and whole-body protein turnover were determined during a 9-h period in a group of seven men while they received hourly isocaloric meals of high-protein (HP) or high-carbohydrate (HC) content. Their responses to feeding were compared with those to a short period of fasting (15-24 h). The 9-h thermic response to the repeated feeding of HP meals was found to be greater than that to the HC meals (9.6 ± 0.6% vs 5.7 ± 0.4% of the energy intake, respectively, t ± SEM, p < 0.01). The rate of whole-body nitrogen turnover over 9 h increased from 17.6 ± 2.2 g on the fasting day to 27.4 ± 1.4 g during HC feeding (NS) and there was a further increase to 58.2 ± 5.3 g resulting from HP feeding (p < 0.001). By using theoretical estimates (based upon ATP requirements) of the metabolic cost of protein synthesis, 36 ± 9% of the thermic response to HC feeding and 68 ± 3% of the response to HP feeding could be accounted for by the increases in protein synthesis compared with the fasting state. Am J Clin Nutr 1990;52:72-80.

KEY WORDS Whole-body protein turnover, protein synthesis, protein breakdown, thermic effect of food, resting energy expenditure, respiratory quotient

Introduction

The well-documented increase in energy expenditure that follows the ingestion of food is generally termed the thermic effect of feeding (TEF). This response varies with the size (1) and the composition of the meal (2), varies among individuals of various genotypes (3), and appears to be reduced in some obese subjects (4, 5). The increase in energy expenditure is due primarily to the obligatory metabolic costs of the processing and storage of the ingested nutrients, but part remains unaccounted for and is termed the facultative component of the response.

Traditionally, TEF was associated with the protein content of the meal and was called the specific dynamic action (6). Although thermogenic responses are also induced by pure carbohydrate and fat administration (7), there is evidence to show that the rate of energy expenditure after meals of protein is higher and that this increase is more prolonged than the responses to isocaloric meals of fat or carbohydrate (7-9). The large thermic effect of protein has been attributed to the high costs of peptide-bond synthesis [because whole-body protein synthesis is increased by feeding (10, 11)] together with the costs of ureogenesis and gluconeogenesis (12). In humans there is a significant correlation between the rates of oxygen consumption and protein synthesis in the basal state (13), but it is not known to what extent the magnitude and prolongation of the TEF reflect the energy cost of the synthesis of tissue protein that is occurring during absorption (14).

The aim of this study was to determine the rates of whole-body protein turnover and energy expenditure during a period of fasting and during periods of feeding either high-carbohydrate or high-protein meals. We also examined the relationship between the changes in the rates of protein synthesis and energy expenditure during two different thermic responses.

Subjects and methods

Subjects

Seven healthy, normal-weight men were recruited for the study. Their physical characteristics are given in Table 1. The experimental protocol was approved by the Ethical Committee of the University of Lausanne and was explained in detail to each subject before he agreed to participate.

Experimental protocol

The study consisted of 3 test days, identical in design and differing only in the composition of the meals given: high carbohydrate (HC, 70% of energy provided), high protein (HP, 70%), or acaloric (flavored water). Each subject completed all 3 test days. The order of presentation of the days was randomized and they were separated by 1-wk intervals. Each test included a measurement of resting energy expenditure (in the postabsorptive state) (RMR) followed 2 h later by the measurements of the rates of protein turnover and energy expenditure during a period of 9 h. The high-carbohydrate and high-protein

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Table 1
Characteristics of the subjects

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y)</td>
<td>25.4 ± 0.8</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>67.6 ± 4.1</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>176.7 ± 3.6</td>
</tr>
<tr>
<td>Body fat content (%)</td>
<td>13.4 ± 1.3</td>
</tr>
<tr>
<td>Total body water (L)</td>
<td>41.8 ± 1.8</td>
</tr>
</tbody>
</table>

* x ± SEM; n = 7.
† Determined from four skinfold thicknesses by the method of Dunnin and Womersley (15).
‡ Estimated from anthropometric measurements (16).

The subjects provided 25 kcal/kg body wt during the 9-h period (equivalent to 2.7 x RMR) and consisted of a series of 11 equicaloric liquid-formula meals made as 70% high-protein powder (Alburone, Sopharga Laboratories, Puteaux, France) and 15% dextrin maltose (Maltrinex, Sopharga Laboratories) substituted isocalorically, together with 15% lipid. These were flavored with cyclamate and saccharin (Assu grin, Hermes Sustoff SA, Zurich) and essences (McCormick SA, Regensdorf, Zurich, Switzerland). On the accloracal day an equivalent volume of water (containing the same flavorings) was given in place of each of the liquid-formula meals.

Figure 1 shows a summary of the design of each test day. Subjects were given a standardized meal (52% carbohydrate, 11% protein, and 37% fat) at 1830 on the evening preceding the test. They slept at the institute overnight, and after a measurement of their postabsorptive RMR, they were given their first meal at 0730 (−2 h). Thereafter meals were given hourly for 11 h. A single dose of [15N]glycine (125 mg, 99 atom %, American International PLC, Buckingham, England) was taken orally with water together with the third meal (0 h). Urine was collected before and during a 9-h period after isotope administration and all postdose urine samples were pooled. The volume of each urine sample was immediately determined and the sample was then acidified with HCl (6 mol/L) to pH 1. Two blood samples were taken, one immediately before the isotope dose (0 h) and the second at the end of the 9-h period (9 h) for the determination of blood urea nitrogen concentration and its isotope enrichment. The reproducibility of the single-dose technique with [15N]glycine as tracer ranged from 3% to 6% when the arithmetic average was used. From the third meal (0 h) on, energy expenditure was measured continuously for 35 min/h (thus nine periods total). Metabolic rate was determined by indirect calorimetry using a ventilated-hood system as previously described (17). The reproducibility of our calorimetric system expressed as the coefficient of variation (CV) of repeated 5-min periods was 1−2%. Subjects remained seated throughout the study period and during the baseline measurement. Additional blood samples were taken at 0, 3, 6, and 9 h for determination of plasma glucose, free fatty acids (FFAs), and insulin concentrations. Serum thyroid hormone concentrations were measured in blood samples taken at 9 h.

Assessment of protein turnover

Protein turnover was estimated from isotope enrichment of urinary and plasma urea and urinary ammonia after a single dose of [15N]-labeled glycine, as described by Fern et al. (18). The [15N] enrichments of urea and ammonia in samples collected during a 9-h period (postdose) on each test day were corrected for background (predose) isotopic enrichment. The rate of whole-body nitrogen turnover (Q; g N/9 h) is calculated by using the equation given by Waterlow et al (19):

\[
Q = E \cdot d/e
\]

where E is the rate of excretion of the end product (g N/9 h), d is the dose of [15N] given (in g), and e is the amount of isotope in the end product (in g). For urea the values of E and e are corrected for the amount of urea and its enrichment, respectively, remaining in the body pool at 9 h (18). The concentration of urea is considered to be that of plasma water and its volume of distribution, equivalent to total body water. Total nitrogen excretion values are similarly corrected for changes in the size of the body pool of urea during the 9-h period.

An arithmetic mean is calculated from the individual values of nitrogen turnover derived from the two end products (ammonia and urea). The end-product average is considered to represent a more accurate estimate of whole-body nitrogen flux than either value given by the individual end products because they are derived from different metabolic pools of nitrogen (20):

End-product average (AA) = (Qa + Qa)/2

where Qa and Qa are the turnover rates derived from urea and ammonia, respectively. Rates of protein synthesis and degradation are calculated according to the following equation:

\[
Q = \text{synthesis} + \text{excretion} = \text{degradation} + \text{intake}
\]

The value of 6.25 was used to convert nitrogen values to protein.

Determination of isotope enrichment

Urinary ammonia and urea were separated at room temperature by the aeration of alkaline urine (pH 12). The ammonia evolved was collected over a 3-h period into a trap containing 10 mL dilute sulphuric acid (21). The ammonia-free urine was treated with 5 U urease (pH 6.5, 37°C for 3 h), and the ammonia liberated was collected by aeration as above. Plasma samples were treated with an equal volume of trichloroacetic acid (200 mL/L) and centrifuged for 15 min at 2000 × g. The pH of the supernatant was increased (to pH 12) and samples were then placed in a boiling water bath for 1 h. They were subsequently treated as urinary urea samples. Samples of sulphuric acid containing trapped ammonia were treated directly with lithium hypobromite to liberate gaseous nitrogen; its isotope enrichment was measured with a gas isotope ratio mass spectrometer (SIRA 10, VG Isogas Instruments, Cheshire, UK).

FIG 1. Experimental design.
Plasma glucose was analyzed by using the glucose oxidase method (glucose analyzer II, Beckman Instruments, Fullerton, CA). Radioimmunoassays were used for the determination of plasma insulin (22) and thyroid hormone concentrations (ARIA II autoanalyzer, Becton Dickinson, Orangeburg, NY). Plasma FFAs were extracted via the method of Dole and Meinertz (23) and determined according to Heindel et al (24). The Berthebot method (25) was used for determination of urinary ammonia concentration (Roche Diagnostica, Berne, Switzerland) and for measurement of plasma and urinary urea concentrations after urease hydrolysis. Urinary urea values were corrected for residual ammonia content. The Kjeldahl method (26) was used for the determination of the total nitrogen in urine and urinary creatinine was measured by using the Jaffe reaction.

Data analysis

Calculation of energy expenditure and substrate oxidation rates were described previously (27). Total energy expenditure (EE) during the 9-h period was derived from interpolated values between the hourly measurements. TEF was calculated as the mean increase in EE during the 9-h period \((\bar{X} EE_{9h})\) above the RMR or above the mean acaloric EE values determined over 9 h and expressed either in relation to RMR or to energy intake \((E_{in})\):

\[
\text{TEF} = \frac{\bar{X} EE_{9h} - \text{RMR}}{\text{RMR}} \times 100
\]

\[
\text{TEF} = \frac{\bar{X} EE_{9h} - \bar{X} \text{ acaloric } EE_{9h}}{E_{in}} \times 100
\]

Data are presented as \(\bar{X} \pm \text{SEM}\). For one subject the urinary collection on the acaloric day was incomplete (as evidenced by low creatinine-nitrogen excretion values) and the values for this day were therefore excluded. Data for protein turnover and substrate oxidations on the acaloric day are presented for six subjects only; all other values are calculated for seven subjects.

Data were analyzed by using a one-way analysis of variance (ANOVA) with repetition for single values and by using a two-way ANOVA with repeated measures for both factors to examine the time courses of the changes occurring during each test day. Individual comparisons were made with a Tukey test for single and repeated measures (28). Each subject acted as his own control.

Results

Energy expenditure and the thermic effect of feeding

The mean changes in the rates of EE during the 9-h period of acaloric, HC, and HP feeding are shown in Figure 2, and the values of the thermic responses are summarized in Table 2. Mean RMR was similar on each occasion but by the first hour of the 9-h period (2 h after the first meal was given), the mean rates of EE of all groups had increased and were significantly higher than resting values \((p < 0.01\) acaloric, \(p < 0.001\) HC and HP). Although the mean rates of EE determined during the 9-h period on the acaloric test days were significantly higher than the RMR values \((p < 0.01\)), the total rates of EE in both fed groups (HC and HP) were significantly greater during the 9-h period than values determined on the acaloric day \((p < 0.001\) at all times). Overall, the thermic response to HP feeding was larger than that to HC feeding \((p < 0.01\)). The mean EE of the fasting and HC groups remained in a steady state during the 9-h period whereas EE in the HP group progressively increased with time as shown in Figure 2. From 5 h on, mean EE during the HP day was significantly greater than that during the HC day \((p < 0.05\)).

Respiratory quotient and rates of substrate oxidation

The mean time courses of the changes occurring in total respiratory quotient \((RQ)\) and rates of fat and carbohydrate oxidation during the 3 test days are shown in Figure 3. Resting (postabsorptive) RQ was similar on each occasion (acaloric 0.848 \(\pm\) 0.017, HC 0.842 \(\pm\) 0.011, and HP 0.861 \(\pm\) 0.011). Two hours after the first meal (at the start of the 9-h period), the mean RQ values of the HC and HP groups had changed to closely reflect the RQ of the food provided, the highest values being seen in the HC group \((p < 0.01\), compared with HP and acaloric values at all times).

Throughout the study period the RQ values remained in a
steady state during the feeding days (HC 0.918 ± 0.007 and HP 0.815 ± 0.006). By contrast the mean RQ on the acaloric day was found to decrease progressively with the increasing duration of the fast (p < 0.05), and mean 9-h values (0.778 ± 0.010) were lower than those determined in either of the fed states (acaloric vs HP, p < 0.01).

The mean rates of substrate oxidation determined in the resting (postabsorptive) state were similar on each test day. During the 9-h period on the acaloric test day, the rate of carbohydrate oxidation progressively decreased and the rate of fat oxidation increased together with the lengthening duration of the fasting period (p < 0.05); overall, the rate of fat oxidation was significantly higher than in either of the two fed states (p < 0.001). During HC feeding the rate of carbohydrate oxidation was greater than that determined during the acaloric or HP days (p < 0.001 at all times), and fat oxidation was reduced to rates significantly lower than those determined during the resting postabsorptive period (p < 0.001). On the HP day, rates of carbohydrate oxidation were reduced when compared with postabsorptive rates determined on that test day (p < 0.01) and remained low throughout the 9-h period. Rates of protein oxidation during the study period on the acaloric test day were similar to values determined during the resting period (45 and 35 mg/min, respectively), whereas during HC feeding there was a significant increase in the rate of protein oxidation from a mean postabsorptive value of 26 to 74 mg/min (p < 0.05). On the HP test day there was a sixfold increase in the rate of protein oxidation during the feeding period (216 mg/min) over resting postabsorptive values (35 mg/min, p < 0.001).

Substrate balances are given in Table 3. All subjects were in positive energy balance on the 2 feeding days. During the acaloric period (15–24 h after the last meal) the major energy source was body fat stores, providing 66% of the calories used. On the feeding days the major substrate oxidized was carbohydrate on the HC day and protein on the HP day, accounting for 69% and 60%, respectively, of the calories used. Because subjects were in positive energy balance, there was a net storage of nutrients, the pattern of which reflected the composition of meals given. During HC feeding, 73% of the energy stored was in the form of carbohydrate whereas during HP feeding protein storage accounted for 75% of the positive energy balance. There was a small net storage of fat and protein on the HC day and of fat and carbohydrate on the HP day.

### Nitrogen excretion

Table 4 gives the mean rates of urinary nitrogen excretion on the 3 test days. Total nitrogen excretion was significantly greater during the HC feeding day than during the acaloric day (p < 0.05) and was greatly increased during the HP feeding day (p < 0.01). Although increases in ammonia and urea nitrogen

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**TABLE 3**

<table>
<thead>
<tr>
<th>Substrate balance during the 9-h study period for the 3 test days*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
</tr>
<tr>
<td>--------------------------------</td>
</tr>
<tr>
<td>Carbohydrate intake</td>
</tr>
<tr>
<td>Carbohydrate oxidation</td>
</tr>
<tr>
<td>Carbohydrate balance</td>
</tr>
<tr>
<td>Fat intake</td>
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<tr>
<td>Fat oxidation</td>
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<td>Fat balance</td>
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<tr>
<td>Protein intake</td>
</tr>
<tr>
<td>Protein oxidation</td>
</tr>
<tr>
<td>Protein balance</td>
</tr>
</tbody>
</table>

* x ± SEM; n = 7.
excretion were found during the HC day compared with the acaloric day, these differences failed to achieve statistical significance. On the HP day both showed marked increases (p < 0.01 compared with HC day).

Rates of whole-body nitrogen turnover and protein synthesis

The individual values for whole-body nitrogen turnover determined from the isotope enrichment of urea and ammonia and the relationship between the two estimates are shown in Figure 4 and the mean values are given in Table 5. On the acaloric and HC test days, values from urea enrichment were greater than those from ammonia (p < 0.05) but there was no consistent pattern found in the relationship between the values determined on the HP day. Whole-body protein turnover was increased above fasting values for all subjects for the HC meals fed conditions (HC 64 ± 17% and HP 1 9 1 ± 43%), although there was a tendency for protein degradation values to fall (NS; increase 0.01 compared with HC day). On the HP day both showed marked increases (p < 0.05) whereas on the acaloric (p < 0.01) and HP (p < 0.05) days. During HP feeding insulin concentrations were intermediate between HC and acaloric values and showed a trend to increase with time (p < 0.05) whereas on the acaloric and HC days, insulin concentrations remained in an apparent steady state. Plasma glucose concentrations reflected the pattern of plasma insulin concentrations during the different test days, being highest during HC feeding (p < 0.01 compared with HP and fasting days), lowest during the acaloric day.

### Table 4

<table>
<thead>
<tr>
<th>Urinary nitrogen excretion during the 9-h study period for the 3 test days*</th>
<th>Acaloric</th>
<th>HC</th>
<th>HP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total nitrogen (mg/9 h)†</td>
<td>3687 ± 355§</td>
<td>6366 ± 494§</td>
<td>19 202 ± 1003</td>
</tr>
<tr>
<td>Urea nitrogen (mg/9 h)†</td>
<td>2971 ± 370§</td>
<td>5351 ± 359§</td>
<td>17 865 ± 955</td>
</tr>
<tr>
<td>Ammonia nitrogen (mg/9 h)</td>
<td>308 ± 28§</td>
<td>392 ± 35§</td>
<td>710 ± 62</td>
</tr>
<tr>
<td>Creatinine nitrogen (mg/9 h)</td>
<td>275 ± 24</td>
<td>266 ± 21</td>
<td>266 ± 20</td>
</tr>
</tbody>
</table>

* x ± SEM; n = 7 for HC and HP, n = 6 for acaloric.
† Values are corrected for changes in body urea pool size during the 9-h period.
§ Significantly different from HC and HP, p < 0.05.
<< Significantly different from HP, p < 0.01.

### Table 5

| Whole-body nitrogen turnover (Q) and rates of protein synthesis and degradation on different test days* |
|----------------------------------------------------------|-----------------|-----------------|-----------------|
| Q ammonia (g N/9 h)                                      | Acaloric        | HC              | HP              |
|                                                        | 16.2 ± 1.7†     | 25.4 ± 1.0†     | 58.8 ± 5.6      |
| Q urea (g N/9 h)                                         | 19.1 ± 3.1†     | 29.3 ± 2.0†     | 57.6 ± 6.0      |
| End-product average (g N/9 h)                           | 17.6 ± 2.2†     | 27.4 ± 1.4†     | 58.2 ± 5.3      |
| Protein intake (g/h)                                     | —               | 62.4 ± 3.6      | 291.2 ± 17.2   |
| Protein synthesis (g/9 h)                               | 87.2 ± 13.0†    | 131.2 ± 6.9†    | 243.9 ± 28.9   |
| Protein degradation (g/9 h)                             | 110.2 ± 13.7    | 109.8 ± 6.1     | 78.9 ± 31.3    |

* x ± SEM; n = 7 for HC and HP, n = 6 for acaloric.
† Significantly different from HP, p < 0.001.

Blood variables

Figures 6, 7, and 8 show the mean plasma concentrations of insulin, glucose, and FFA determined during the 9-h period on each of the test days (0 h is 2 h after the first meal). Significantly greater plasma insulin concentrations were found at all times during HC feeding as compared with the acaloric (p < 0.01) and HP (p < 0.05) days. During HP feeding insulin concentrations were intermediate between HC and acaloric values and showed a trend to increase with time (p < 0.05) whereas on the acaloric and HC days, insulin concentrations remained in an apparent steady state. Plasma glucose concentrations reflected the pattern of plasma insulin concentrations during the different test days, being highest during HC feeding (p < 0.01 compared with HP and fasting days), lowest during the acaloric day.
and intermediate on the HP day (overall $p < 0.05$ compared with fasting day). There was a tendency for plasma glucose concentrations to fall with the increasing duration of the fasting period (NS). In both fed groups (HP and HC), plasma FFA concentrations remained low in comparison with acaloric values ($p < 0.01$ at all times) and there were no significant changes during the 9-h period. On the acaloric day FFA concentrations showed a marked increase with time during the fasting period ($p < 0.001$). Serum thyroid hormone concentrations were determined at the end of the period on each test day. The mean concentrations of serum free $T_3$ and total $T_4$ were similar for each of the 3 test days [$T_3$ values (nmol/L): acaloric $1.8 \pm 0.1$, HC $1.6 \pm 0.1$, and HP $1.8 \pm 0.1$; $T_4$ values (nmol/L): acaloric $113 \pm 8$, HC $112 \pm 11$, and HP $110 \pm 10$]. In the six subjects studied, we observed no correlation between the rates of whole-body protein turnover in the fasting state and thyroid status.

**Discussion**

*Energy expenditure and the thermic effect of feeding*

This study compares the metabolic responses to feeding iso-caloric meals of HP or HC content with those to a short period of fasting. During the 9-h period on the acaloric day, the mean rate of EE for each subject was found to be 9.7% higher than the resting value (determined that morning). This may be attributed to the subjects being more alert and to some degree of body movement later in the day as well as the additional stress of fasting for a prolonged period of time. Assuming that the same changes are present during the feeding days, it seems appropriate to use the mean EE of the acaloric day as the control baseline value for the calculation of TEF. TEFs resulting from HC and HP feedings during the 9-h period were $5.7 \pm 0.4\%$ and $9.6 \pm 0.6\%$ of the energy intake, respectively. A large thermic response after a single meal of protein was reported and was shown to be due to both a larger net increase in EE and a response more prolonged than the response to carbohydrate or to fat (7, 9). Although the total (9-h) thermic response to the repeated feeding of HP meals was greater than that to HC meals, the difference between the meal types was evident only from the fifth hour onwards (7 h after the first meal). EE continued to increase throughout the 9-h period on the HP day (in contrast to the apparent steady-state EE observed on the acaloric and HC test days) and reached the highest value during the eighth and ninth hours. The magnitude of the thermic responses assessed from hourly feeding cannot be compared with that reported after a single meal (29, 30) because the overall response cannot be completely integrated: at the end of the study, when the nutrient administration was discontinued, EE was still elevated so that our TEF values are underestimated. In the present study the mean TEF value determined on the HC day was similar to that found by Zurlo et al (31) (4.9%) during continuous enteral perfusion of a mixed formula diet (containing 17% protein energy) administered over a 9-h period at an amount corresponding to $1.4 \times$ RMR.

*Respiratory quotient and rates of substrate oxidation*

On the 2 feeding days the mean RQ closely reflected the RQ of the food provided. A new steady state was achieved (in com-
comparison with resting, postabsorptive values) 2 h after the first meal, as previously shown by Garlick et al (32). Progressive decreases in RQ were observed on the acaloric test day (15–24 h after the last meal), reflecting increasing rates of fat oxidation concomitant with decreasing carbohydrate oxidation. On both feeding days the rates of fat and carbohydrate oxidation remained in an apparent steady state during the study period, and fat oxidation rates were greatly reduced when compared with the fasting state. As expected the major substrates oxidized were carbohydrate on the HC day (69% of EE) and protein on the HP day (60% of EE). In the latter case the RQ observed after feeding was reduced as compared with the prefeeding value and approached that of protein (Fig 3). All subjects were in positive energy balance during the feeding days and the pattern of energy storage reflected the composition of the food eaten.

**Blood variables**

Plasma insulin and glucose concentrations were greatest during HC feeding and lowest during the acaloric day. During the HP day plasma glucose concentrations remained in a steady state during the study period whereas there was a progressive increase in the mean plasma insulin concentrations with time. This may be due to a greater stimulation of insulin secretion by the increasing amino acid flux arising from the large dietary protein intake. It is of interest to note that the mean trend in plasma insulin concentration on HP day was very similar to that seen in the rate of EE, suggesting that insulin concentrations may have played a role in the increasing rate of protein synthesis during the study period. Plasma FFA concentrations reflected the differences in the amounts of fat oxidation between the test days, being greatest during the acaloric test day and increasing with the duration of the fasting period.

**Rates of whole-body protein turnover, synthesis, and degradation**

The relationship between Q values determined from the isotope enrichments of urea and ammonia are shown in Figure 4. During the acaloric and HC days the values determined from urea enrichment were similar to or greater than those from ammonia enrichment (with one exception) and the degree of difference between the end products was similar on both test days. During HP feeding there was no relationship between the two values determined for each subject, but the proportionate differences between the end products were similar to those observed on the other test days.

During the 9-h period on the HP day, subjects received 4.2 g protein/kg (compared with 0.9 g protein/kg on the HC day), which presented a supraphysiological challenge to the subjects. As a result we might have expected to encounter difficulties in the calculation of Q arising from the incomplete mixing of labeled and unlabeled nitrogen because of such a large protein intake. In this case, marked underlabeling of urea could have occurred, which would have generated falsely high values for Q when compared with those calculated from ammonia labeling. Because this did not occur, it appears that the single-dose model is appropriate over a wide range of metabolic conditions and that the values of Q determined on the HP day represent a quantitative estimate of whole-body nitrogen turnover. In contrast the degradation values calculated on the HP day appear to be less reliable because two of the seven values were found to be negative. The accuracy of these values is reliant on the precise knowledge of the nitrogen intake of the subjects during the period of measurement, and it assumes that there is a complete digestion and absorption of the ingested nitrogen within this period. In the present study, meals were made up from formulas and the subjects were supervised at meal times. We therefore have confidence in the values for their nitrogen intake, but it is possible that a substantial fraction of the HP diet remained in the stomach and intestine at the end of the 9-h period. In addition, this unabsorbed fraction may vary greatly between individuals. The subjects commented that they found the HP meals difficult to eat late in the study period because of strong feelings of satiety, but no objective assessment of postprandial satiety was made.

The differences in whole-body nitrogen turnover, protein synthesis, and breakdown between the acaloric and the HC states are similar to those reported by other workers (10, 11, 18). In the present study there were marked increases in Q (65%) and in the rates of protein synthesis (64%) during HC feeding when compared with values determined during a short-term test (15–24 h) whereas rates of degradation did not appear to change. Although the values for whole-body nitrogen turnover obtained in the present study were slightly higher than those reported by other workers (11, 18), the direction and magnitude of the changes occurring from the fasted to the fed states were almost identical. It is possible that our higher values reflect the fact that the subjects were lean young men with a large fat-free mass in relation to body weight. Other studies reported a reduced rate of protein degradation in response to feeding (33) or observed no change (10, 11, 18). The reasons for discrepant results remain to be investigated but may be related to the method used to estimate protein turnover and to the duration of the study, which influences the rate of isotope recycling.

In the present study there were very large increases in the rates of Q and protein synthesis on the HP day as compared with the fasting state and with the HC test day. It appears that there was a tendency for the rates of degradation to fall during the HP day, but the range of values was very large and hence the difference was not statistically significant.

**Contribution of whole-body protein synthesis to energy expenditure**

One aim of this study was to attempt to quantify the contribution of protein synthesis to total EE in the fasting and fed states. An estimation of the theoretical costs of protein synthesis based on biochemical principles (assuming that 4 mol ATP and/or GTP are utilized in the synthesis of each peptide bond) gives a value of 0.67 kcal/g protein synthesized as the theoretical minimum energy requirement (34). Other workers made allowances for the ATP costs of amino acid uptake and RNA synthesis, which increase the estimated costs to 0.86 kcal/g (13) and 1.00 kcal/g, respectively (12). By use of the minimum estimated costs (0.67 kcal/g), our rates of protein synthesis would theoretically account for 8.7% of the total EE in the fasting state, 11.7% during HC feeding, and 19.8% during HP feeding. Our results thus differ from those of Garlick (34), where a constant contribution (8%) of protein synthesis to EE in both fasted and fed states was observed when subjects received a normal diet. By use of the same theoretical value of costs of protein synthesis, the mean contribution made by the increment in protein synthesis to the increment in EE (ie, when the HC and
HP days were compared with the acaloric day) was 36 ± 9% on the HC day and 68 ± 3% on the HP day. The increased rates of ureogenesis in the fed state compared with the fasting state represents an additional cost. Using the theoretical value of 4 ATP/mol urea synthesized given by Flatt (12), the relative contribution made by the increment in urea synthesis to the thermic responses would average 9 ± 1% on the HC day and 28 ± 3% on the HP day. The combined effect of increased protein synthesis and nitrogen excretion would thus account for a total of 45 ± 10% of the HC TEF and 96 ± 15% of the HP TEF. During HC feeding the remaining 55% of the increased thermogenesis that is not accounted for may be due to the costs of glycogen storage (35) and possibly to some degree of stimulation of the sympathetic nervous system (7).

The above calculations are based upon the theoretical estimates of the costs of protein synthesis, and care should be taken about its interpretation because of the small number of subjects and the errors inherent in the methodology used (36). Although it appears that the TEF during HP feeding may be entirely accounted for by the processing and storage of the large dietary protein intake, the range of individual values was large (69–150%), and no allowance was made for the processing of other ingested nutrients (e.g., carbohydrate and fat) or for changes in the rates of gluconeogenesis.

Experimental data based on the physiological approach can be used to illuminate the issue of the metabolic cost of protein synthesis, by establishing the regression between the rates of protein synthesis and EE (37). This tends to give values that are greater than those calculated according to the biochemical approach. Part of the discrepancy may be explained by other energy-requiring processes, which necessarily proceed in parallel with protein synthesis and which may increase the energy requirement by as much as 50%, that have not been included in the value of 0.67 kcal/g. Furthermore, it is possible that current methodology underestimates the true rates of protein synthesis because of an inability to assess the exact contribution of rapidly turning over proteins, as suggested by Jackson et al (38).

Estimates of the experimental cost of protein synthesis can be made by comparing the effect of fasting vs feeding on both the rates of EE and protein synthesis. Only the values for the HP diet were considered because those determined on the HC day includes extra energy costs, such as the cost of glycogen storage or lipogenesis. With the HP diet the energy cost of protein synthesis was calculated to be 1.15 kcal/g protein synthesized. When deductions are made for the increased cost of ureogenesis on the feeding day (12), the value is reduced to 0.84 kcal/g protein synthesized. This appears to be very close to theoretical estimates based upon biochemical principles.

In conclusion, the present study shows that the repeated feeding of meals of a high-protein content causes a thermic response that is greater than that of high-carbohydrate isocaloric meals. By use of theoretical estimates of the metabolic costs of protein synthesis, it appears that 36% of the TEF response to feeding high carbohydrate meals may be accounted for by the postprandial increase in protein synthesis. During the thermic response to high-protein feeding, this value is greater and accounts for 68% of the increase in energy expenditure. Because the total thermic response to high-protein feeding appears to be largely accounted for by the high costs of protein synthesis and ureogenesis, an estimate of the energy cost of the protein synthesis may tentatively be made. The value thus determined was 0.84 kcal/g protein synthesized, which is very close to theoretical estimates based on biochemical considerations.

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References


