EFFECTS OF PROTEIN DEFICIENCY ON MUSCLE
MYOFIBRILLAR PROTEIN TURNOVER IN
ADULT RATS

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Summary The rates of gain, catabolism, synthesis and reutilization of
myofibrillar protein were measured in adult rats fed a protein-free diet,
low protein diet (2% lactalbumin) or control diet (10% lactalbumin) for
14 to 31 days. Two forms of synthesis were measured: exogenous
synthesis (nitrogen derived from diet) and endogenous synthesis (nitrogen
derived from catabolized body protein).
The rate of gain of myofibrillar protein was measured as the rate of
increase in its weight and the rate of catabolism was determined from
urinary 3-methylhistidine excretion. The rate of total synthesis was
calculated as the sum of these two rates. Exogenous synthesis was
calculated from the recovery of isotope in protein 24 hr after oral
administration of $^{15}$N-leucine and endogenous synthesis was calculated as
the difference between the total synthesis and exogenous synthesis.
Reutilization was calculated as the ratio of endogeneous synthesis to
catabolism.
The rate of catabolism was slightly decreased in protein deficiency (2.1,
2.1 and 2.6% in the protein-free, low protein and control groups,
respectively), while that of synthesis was significantly decreased in protein
deficiency (1.3, 2.0 and 3.3% in the respective groups). Restriction of
protein intake resulted in a decrease in the rate of exogenous synthesis,
without appreciable change of endogenous synthesis. The reutilization
rate of endogenous N was estimated to be about 70% in rats with
restricted protein intakes and about 50% in those with a normal protein
intake.

Keywords protein deficiency, myofibrillar protein, 3-methylhistidine,
$^{15}$N-leucine, protein synthesis, catabolism, reutilization

Growth and nitrogen balance of animals are markedly influenced by protein
intake, and there is an intimate relation between protein supply and protein

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metabolism. However, the effects of dietary protein on the quantitative aspects of protein metabolism and on protein metabolism in individual tissues and organs have not been studied sufficiently. This is mainly due to methodological difficulties, such as those in measurements of N reutilization and the metabolic pool size, in studies on protein turnover using isotopes. Recently, the urinary excretion of 3-methylhistidine was found to be a reliable index of the catabolism of myofibrillar protein (1, 2), and there have been studies on the catabolism of myofibrillar protein measured in this way, but few of these were quantitative studies. Therefore, in this work, we estimated the catabolic rate of myofibrillar protein quantitatively by measuring urinary 3-methylhistidine excretion and the concentration of this amino acid in myofibrillar protein. Myofibrils are a major component of muscle that constitutes about 45% of the total body weight (3). Thus, they probably play a significant role in protein metabolism of the whole body. We also examined the effects of protein deficiency on the rate of synthesis of myofibrillar protein, estimating this rate as the sum of the rates of increase and catabolism of myofibrillar protein.

We considered the synthesis as two-part, exogenous and endogenous, on the basis of the origin of the N used for protein synthesis (These will be discussed later). Exogenous synthesis was determined by measuring the incorporation of $^{15}$N into myofibrillar protein during the 24 hr after oral administration of $^{15}$N-leucine. Endogenous synthesis was estimated as the difference between the exogeneous and total rates of synthesis. From these data, we could calculate the reutilization of endogeneous nitrogen, which is difficult to determine by other procedures.

MATERIALS AND METHODS

Animals and diets. Male Sprague-Dawley rats,1 weighing about 300 g, were fed a standard diet containing 12% lactalbumin2 for one week. Then, they were divided into three groups of 15 animals each and fed ad libitum a protein-free, low protein (2% lactalbumin) or control (10% lactalbumin) diet, respectively, for 14 to 31 days. The compositions of the diets are shown in Table 1. The animals were kept in separate cages in an air-conditioned room at 22±2°C. Body weight and food intake were measured daily at 10:00 a.m. For three 3-day periods (days 18 to 20, 22 to 24 and 28 to 30), the animals were kept in individual metabolic cages and their total urine and feces were collected. At 10:00 a.m. on days 14, 22 and 31, 5 rats from each group were anesthetized with ether and blood was withdrawn by cardiac puncture into a heparinized syringe. The gastrocnemius muscle was rapidly removed and stored at −70°C for analysis. The viscera, skin with hair and adipose tissue were removed, and the rest of the carcass was heated at 120°C for 20 min in an autoclave. The muscle then was separated from the skeleton. The total weight of

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1 Sprague-Dawley: From Japan Keary Co., Ltd., Osaka, Japan.
2 Lactalbumin: From Sigma Chemical Co., St.Louis, Mo., U.S.A.

**Table 1. Compositions of diets.**

<table>
<thead>
<tr>
<th></th>
<th>Protein-free diet</th>
<th>Low protein diet</th>
<th>Control diet</th>
<th>Standard diet</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(g/kg)</td>
<td>(g/kg)</td>
<td>(g/kg)</td>
<td>(g/kg)</td>
</tr>
<tr>
<td>Lactalbumin</td>
<td>0</td>
<td>20</td>
<td>100</td>
<td>120</td>
</tr>
<tr>
<td>α-Starch</td>
<td>578</td>
<td>565</td>
<td>511</td>
<td>498</td>
</tr>
<tr>
<td>Sucrose</td>
<td>289</td>
<td>282</td>
<td>256</td>
<td>249</td>
</tr>
<tr>
<td>Oil</td>
<td>50</td>
<td>50</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>Salt mixture&lt;sup&gt;a&lt;/sup&gt;</td>
<td>50</td>
<td>50</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>Cellulose</td>
<td>20.5</td>
<td>20.5</td>
<td>20.5</td>
<td>20.5</td>
</tr>
<tr>
<td>Vitamin mixture&lt;sup&gt;b&lt;/sup&gt;</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Choline chloride</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Chocola A&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.4</td>
<td>0.4</td>
<td>0.4</td>
<td>0.4</td>
</tr>
<tr>
<td>dl-α-Tocopherol</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
</tr>
</tbody>
</table>

<sup>a</sup> Harper Mixture, obtained from Oriental Kobo Co., Tokyo, Japan, containing the following percentages of salts: calcium carbonate, 29.29; calcium phosphate, dibasic, 0.43; potassium phosphate, monobasic, 34.31; sodium chloride, 25.06; magnesium sulfate, 9.98; ferric citrate, 0.623; cupric sulfate, 0.156; manganese sulfate, 0.121; zinc chloride, 0.02; potassium iodide, 0.0005; ammonium molybdate, 0.0025.

<sup>b</sup> Harper Mixture, obtained from Oriental Kobo Co., containing the following percentages of vitamins: thiamine•HCl, 0.059; riboflavin, 0.059; nicotinic acid, 0.294; calcium pantothenate, 0.235; pyridoxine•HCl, 0.029; vitamin K, 0.006; d-biotin, 0.001; folic acid, 0.002; vitamin B₁₂, 0.0002; inositol, 1.176; ascorbic acid, 0.588; lactose, 97.551.

<sup>c</sup> From Eisai Co., Tokyo, Japan. The preparation contained 30,000 IU vitamin A and 75 µg vitamin D₂ per ml.

Skeletal muscles was calculated as the difference between the weights of the carcass without viscera, skin and adipose tissue and the skeleton.

**Analyses.** The myofibrillar protein was fractionated by the method of Goldberg (<sup>4</sup>) with slight modifications. Gastrocnemius muscle weighing 350 mg was minced with scissors and homogenized in 15 ml of cold buffer of low ionic strength (0.01 M phosphate, pH 7.4) in a glass homogenizer with a teflon pestle. The homogenate was centrifuged at 3,000 rpm for 20 min to separate the soluble fraction (amino acids and sarcoplasmic protein) and insoluble fraction (myofibrillar and connective tissue protein). The insoluble fraction was mixed with 10 ml of 0.3 N NaOH, stood overnight and then centrifuged to separate myofibrillar protein (in the supernatant) from connective tissue protein (in the precipitate).

For measurement of 3-methylhistidine in myofibrillar protein, the gastrocnemius muscle was hydrolyzed with 6N HCl at 110°C for 24 hr, and 3-methylhistidine in a portion of the hydrolysate was measured in an automatic

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amino acid analyzer, as described by Long et al. (5). Urinary 3-methylhistidine excretion was measured in the urine collected from days 22 to 24. Before analysis, urine samples were heated with an equal volume of 12 N HCl at 110°C for 2 hr to convert n-acetyl-3-methylhistidine to 3-methylhistidine (6).

The animals on a low protein diet and control diet killed on day 22, were administered $^{15}$N-leucine (95 atom %) including 10 mg of $^{15}$N 24 hr before the autopsy. Although it was desirable that the tracer for dietary amino acids is administered with the diet, the rats were force-fed the tracer as 2.5 ml of saline solution (2.012 mg $^{15}$N/ml) twice during an interval of one hour between the treatment, since rats in a pilot study did not consume a diet containing an adequate amount of $^{15}$N-leucine for tracing. This treatment at 10:00-11:00 a.m., however, can be expected to give a similar effect as administering the tracer with the diet, since it was confirmed that a part of the diet still remained in their gastrointestinal tract at that time of ad libitum feeding. For analysis of $^{15}$N, nitrogen in the samples was converted to ammonium sulfate by the method of Kjeldahl, and a portion was used for preparing a discharge tube with vacuum attachment according to the method of Dumas, and subjected to optical spectrographic analysis (7).

Nitrogen of the urine, feces and muscle fractions was analyzed by the method of Kjeldahl.

**CALCULATION**

The rate of catabolism of myofibrillar protein (C) was calculated from the urinary excretion of 3-methylhistidine (U-3-MH) and the concentration of 3-methylhistidine in the myofibrillar protein (3-MH/MFP) using the following equation:

$$C = 0.8 \cdot \frac{U-3-MH}{(3-MH/MFP)}$$

(a)

The coefficient of 0.8 was chosen to represent the contribution of muscle 3-methylhistidine to total urinary 3-methylhistidine (8), for the reasons described in the discussion. The rate of gain of myofibrillar protein (G) was calculated from the body weights on days 22 and 31 (BW$^{22}$, BW$^{31}$) in rats fed for 31 days, the ratio of muscle weight to body weight (MW/BW) and the myofibrillar protein concentration in the muscle (MFP/MW) as follows:

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4 $^{15}$N-leucine: From Hikari Kogyo Co., Ltd., Tokyo, Japan.

5 Vacuum attachment: NIA-1 type, Sample discharge preparing devise, Japan Spectroscopic Co., Ltd., Tokyo, Japan.

6 Optical Spectrographic analysis: NIA-1 type, N-15 Analyzer, Japan Spectroscopic Co., Ltd., Tokyo, Japan.

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Since the rate of gain is the difference between the rates of synthesis and catabolism, the rate of synthesis ($S$) was estimated as the sum of the catabolic rate and growth rate obtained from (a) and (b), respectively.

$$S = G + C \quad (c)$$

Nitrogen for protein synthesis is derived from both dietary and endogenous sources, so the rate of synthesis is the sum of the rates of exogenous synthesis ($S_{ex}$) and endogenous synthesis ($S_{en}$).

$$S = S_{ex} + S_{en} \quad (d)$$

The exogenous synthesis was estimated by multiplying the N intake ($N_{in}$) by the rate of recovery of $^{15}$N in myofibrillar protein 24hr after administration of $^{15}$N-leucine ($^{15}$N_{MFP}/^{15}N_{admin}$).

$$S_{ex} = N_{in} \cdot ^{15}N_{MFP}/^{15}N_{admin} \quad (e)$$

The endogenous synthesis was calculated from formulae (c), (d) and (e). The rate of reutilization of endogenous N was estimated as the ratio of the rates of endogenous synthesis and catabolism.

### RESULTS

#### Food intake and changes in body weight

The food intake of the control group was nearly constant throughout the experiment, while that of the group on the protein-free diet decreased, rapidly in the first week and then more gradually. The intake of the low protein group ranged from 16 to 21g per day. The mean daily intakes in the control, low protein and protein-free groups during the experiment period were about 22, 18 and 14g, respectively.

Changes in body weight are shown in Fig. 1. The body weight of the control group increased, while that of the protein-free group decreased. The low protein group tended to maintain a steady body weight except in the first week. The mean daily gains were 3.3g in the control group, −1.0g in the low protein group and −3.0g in the protein-free group.

#### N balance

Table 2 shows the results on N intake, urinary and fecal N excretions and N balance of the groups during the 3-day periods from days 18 to 20 and 28 to 30. Although the N intake of the low protein diet group was 40 to 50mg per day, the urinary N of this group was slightly less than that of the protein-free diet group.

Fecal N excretion increased with increase in protein intake. The N balances of
Fig. 1. Changes of body weight in groups fed protein-free, low protein (2% lactalbumin) or control (10% lactalbumin) diets for 14 to 31 days. Values for body weight are means for 15 rats from day 0 to 14, 10 rats from day 15 to 22, and 5 rats from day 23 to 31.

Table 2. Food and N intakes, N excretions and N balance in the three groups.a

<table>
<thead>
<tr>
<th>Diet</th>
<th>n^b</th>
<th>Food intake (g/day)</th>
<th>Intake N (mg/day)</th>
<th>Urinary N (mg/day)</th>
<th>Fecal N (mg/day)</th>
<th>N balance (mg/day)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 18 to 20</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Protein-free</td>
<td>10</td>
<td>13.5 ± 1.2</td>
<td>—</td>
<td>43.2 ± 3.6</td>
<td>19.6 ± 4.0</td>
<td>—2 ± 6.4</td>
</tr>
<tr>
<td>Low protein</td>
<td>10</td>
<td>19.9 ± 2.4</td>
<td>50.1 ± 6.0</td>
<td>42.7 ± 4.2</td>
<td>28.0 ± 4.3</td>
<td>—20.6 ± 7.1</td>
</tr>
<tr>
<td>Control\d</td>
<td>10</td>
<td>22.0 ± 1.3</td>
<td>277.0 ± 16.5</td>
<td>110.3 ± 16.3</td>
<td>49.8 ± 6.3</td>
<td>116.8 ± 19.5</td>
</tr>
<tr>
<td>Day 28 to 30</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Protein-free</td>
<td>5</td>
<td>11.7 ± 0.9</td>
<td>—</td>
<td>33.0 ± 5.7</td>
<td>17.2 ± 1.9</td>
<td>—50.1 ± 7.6</td>
</tr>
<tr>
<td>Low protein</td>
<td>5</td>
<td>16.4 ± 0.9</td>
<td>41.3 ± 2.3</td>
<td>30.6 ± 4.7</td>
<td>24.6 ± 8.9</td>
<td>—14.3 ± 12.0</td>
</tr>
<tr>
<td>Control</td>
<td>5</td>
<td>21.0 ± 1.6</td>
<td>263.8 ± 20.5</td>
<td>102.3 ± 20.1</td>
<td>45.3 ± 11.1</td>
<td>116.2 ± 23.6</td>
</tr>
</tbody>
</table>

a Means ± SD.  b Number of rats.  c 2% lactalbumin diet.  d 10% lactalbumin diet.

the respective groups were similar in the period from day 18 to 20 and in that from day 28 to 30. The groups on protein-free and low protein diets showed negative balances of about —60 and —20 mg N, respectively, while the control group showed a positive balance of 120 mg N.

Gain of myofibrillar protein

The weights of muscle as percentages of the total body weight of rats killed on days 14, 22 and 31 are shown in Table 3. The values in the protein-free and low protein groups were slightly higher than that of the control group, but in all groups
Table 3. Weights of muscle as percentages of the total body weight.

<table>
<thead>
<tr>
<th>Day</th>
<th>Protein-free diet (%)</th>
<th>Low protein diet (%)</th>
<th>Control diet (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>14</td>
<td>43.9 ± 0.9</td>
<td>44.6 ± 2.5</td>
<td>41.7 ± 1.4</td>
</tr>
<tr>
<td>22</td>
<td>44.5 ± 1.7</td>
<td>43.2 ± 2.9</td>
<td>42.8 ± 3.3</td>
</tr>
<tr>
<td>31</td>
<td>43.4 ± 1.2</td>
<td>42.6 ± 2.2</td>
<td>39.9 ± 1.1</td>
</tr>
</tbody>
</table>

Mean:

- Mean ± SD for 5 rats.
- Mean of values on days 14, 22 and 31.

The percentage of muscle did not change during the experiment. The average value of days 14, 22 and 31 was 43.9% in the protein-free group, 43.5% in the low protein group and 41.5% in the control group.

Results on the concentrations of myofibrillar protein are shown in Table 4. Values were about 20 mg N per g muscle (61% of the total muscle protein), irrespective of the degree or duration of protein deficiency. These results are in agreement with the findings of Young (3).

Consequently, the total amount of myofibrillar protein was proportional to the body weight. The gain of myofibrillar protein between days 22 and 31 was calculated from the concentration of myofibrillar protein, and the body weight and values are shown in Table 5. The daily gains of myofibrillar protein were -17.5, -3.9 and 23.3 mg N in the protein-free, low protein and control groups, respectively.

Catabolism of myofibrillar protein

Table 6 shows the urinary excretion of 3-methylhistidine released from skeletal muscle (80% of the total urinary excretion (8)), the concentration of 3-methylhistidine in myofibrillar protein and the absolute amount and the fractional rate of catabolism of myofibrillar protein. The concentration of 3-methylhistidine was about 0.033 μmole per mg N of myofibrillar protein in all groups. The absolute amounts of catabolism, calculated by dividing the urinary excretion of 3-methylhistidine by the 3-methylhistidine concentration in myofibrillar protein, were 49.6, 57.5 and 91.2 mg N per day in the protein-free, low protein and control diet groups, respectively.

The fractional catabolic rates, which are the ratios of the total amount of catabolized protein to the mass of myofibrillar protein, were 2.08% in the protein-free group, 2.10% in the low protein group and 2.60% in the control group. The values were slightly lower in protein deficiency, but the differences from that of the
<table>
<thead>
<tr>
<th>Protein-free diet</th>
<th>Low protein diet</th>
<th>Control diet</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Myofibrillar protein</td>
<td>Muscle protein</td>
</tr>
<tr>
<td></td>
<td>(mg N/g muscle)</td>
<td>(mg N/g muscle)</td>
</tr>
<tr>
<td>Days of exp.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>14</td>
<td>20.3 ± 0.9§</td>
<td>32.2 ± 0.7</td>
</tr>
<tr>
<td>22</td>
<td>21.2 ± 0.8</td>
<td>33.5 ± 0.7</td>
</tr>
<tr>
<td>31</td>
<td>19.4 ± 0.6</td>
<td>32.5 ± 0.7</td>
</tr>
<tr>
<td>Mean*</td>
<td>20.3 (61%)</td>
<td>32.2 (100%)</td>
</tr>
</tbody>
</table>

* From gastrocnemius muscle. § Crude protein of gastrocnemius muscle. * Mean ± SD for 5 rats. * Mean of the values on days 14, 22, and 31. § Protein as percentage of total muscle protein.
Table 5. Gain of myofibrillar protein from day 22 to 31.

<table>
<thead>
<tr>
<th>Diet</th>
<th>Body weight&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Total myofibrillar protein&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Gain/day</th>
<th>Fractional rate&lt;sup&gt;c&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Day 22 (g)</td>
<td>Day 22 (mg N/rat)</td>
<td>Day 31</td>
<td>Day 31 (mg N/day)</td>
</tr>
<tr>
<td>Protein-free</td>
<td>270 ± 12&lt;sup&gt;1,4&lt;/sup&gt;</td>
<td>2,394 ± 105&lt;sup&gt;1&lt;/sup&gt;</td>
<td>252 ± 14&lt;sup&gt;1&lt;/sup&gt;</td>
<td>2,237 ± 126&lt;sup&gt;1&lt;/sup&gt;</td>
</tr>
<tr>
<td>Low protein</td>
<td>312 ± 14&lt;sup&gt;2&lt;/sup&gt;</td>
<td>2,742 ± 125&lt;sup&gt;2&lt;/sup&gt;</td>
<td>308 ± 11&lt;sup&gt;2&lt;/sup&gt;</td>
<td>2,707 ± 99&lt;sup&gt;2&lt;/sup&gt;</td>
</tr>
<tr>
<td>Control</td>
<td>418 ± 19&lt;sup&gt;3&lt;/sup&gt;</td>
<td>3,504 ± 160&lt;sup&gt;3&lt;/sup&gt;</td>
<td>443 ± 19&lt;sup&gt;3&lt;/sup&gt;</td>
<td>3,713 ± 157&lt;sup&gt;3&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup> Body weights on days 22 and 31 in rats fed for 31 days.

<sup>b</sup> Calculated by multiplying the body weight on day 22 or 31 in rats fed for 31 days by the percentage weight of muscle (Table 3) and the myofibrillar protein concentration in muscle (Table 4).

<sup>c</sup> Daily gain of myofibrillar protein as a percentage of the total myofibrillar protein on day 22.

<sup>d</sup> Mean ± SD for 5 rats. Means in the same column not sharing a common superscript figure are significantly different (p < 0.05).
Table 6. Catabolism of myofibrillar protein.

<table>
<thead>
<tr>
<th>Diet</th>
<th>Urinary 3-methylhistidine(^a) ((\mu)mole/day)</th>
<th>3-Methylhistidine concentration ((\mu)mole/mg N m.f.p.(^b))</th>
<th>Catabolism(^c) (mg N/day)</th>
<th>Fractional catabolic rate(^d) (%)</th>
<th>Half-life(^e) (day)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protein-free</td>
<td>1.64 ± 0.12(^f)</td>
<td>0.032 ± 0.003(^3)</td>
<td>49.6 ± 3.7(^1)</td>
<td>2.08 ± 0.16(^1)</td>
<td>33.4 ± 2.6(^1)</td>
</tr>
<tr>
<td>Low protein</td>
<td>1.94 ± 0.22(^2)</td>
<td>0.034 ± 0.003(^1)</td>
<td>57.5 ± 6.6(^2)</td>
<td>2.10 ± 0.24(^1,2)</td>
<td>33.4 ± 4.1(^1,2)</td>
</tr>
<tr>
<td>Control</td>
<td>3.07 ± 0.50(^3)</td>
<td>0.034 ± 0.001(^1)</td>
<td>91.2 ± 15.4(^3)</td>
<td>2.60 ± 0.44(^2)</td>
<td>27.2 ± 4.8(^2)</td>
</tr>
</tbody>
</table>

\(^a\) Values were calculated assuming that 80% of the total urinary 3-methylhistidine excretion is derived from myofibrillar protein breakdown in skeletal muscle.

\(^b\) Abbreviation for myofibrillar protein.

\(^c\) Values calculated by dividing urinary 3-methylhistidine excretion by the 3-methylhistidine concentration of myofibrillar protein.

\(^d\) Catabolism of myofibrillar protein as a percentage of catabolism of total myofibrillar protein of skeletal muscle (Table 5).

\(^e\) Half-life calculated as 0.693/fractional catabolic rate.

\(^f\) Mean ± SD for 5 rats. Values in the same column not sharing a common superscript figure are significantly different (\(p < 0.05\)).
control group were not significant. The half-lives of myofibrillar protein, calculated from these fractional rates, were 33 days in the protein-free and low protein diet groups and 27 days in the control diet group.

**Synthesis of myofibrillar protein**

As shown in Table 7, the absolute amounts of synthesis, estimated as the sum of the gains and catabolisms, were 32.1, 53.6 and 114.5 mg N in the protein-free, low protein and control diet groups, respectively. Since the protein-free group showed negative growth and the lowest catabolism of myofibrillar protein, its synthesis was also the lowest, and since the control group showed high values of both gain and catabolism, its synthesis was the highest.

The fractional synthetic rates were 1.34, 1.96 and 3.27%, respectively, in the protein-free, low protein and control diet groups. The rate of synthesis was influenced more than the catabolic rate by the protein intake. Thus, the rate of gain was affected more by the rate of synthesis than by the rate of catabolism.

**Exogenous and endogenous syntheses and reutilization**

Table 8 shows the amounts of exogenous synthesis, calculated by multiplying the N intake by the rate of recovery of $^{15}$N in myofibrillar protein. The recovery rates were 28.0% in the low protein group and 25.6% in the control group. Consequently, the exogenous syntheses were 14.7 and 69.7 mg N per day, respectively, in these groups. The fractional rate was also lower in rats on a low protein diet than in those on a control diet. From these results, it was concluded that the exogenous rate of synthesis was greatly affected by the protein intake.

Values for endogenous synthesis, calculated from the difference between the total synthesis and exogenous synthesis, are shown in Table 9. When expressed as absolute amounts, the values increased with increase in protein intake, but when

<table>
<thead>
<tr>
<th>Diet</th>
<th>Gain&lt;sup&gt;a&lt;/sup&gt; (mg N/day)</th>
<th>Catabolism&lt;sup&gt;b&lt;/sup&gt; (mg N/day)</th>
<th>Synthesis&lt;sup&gt;c&lt;/sup&gt; (mg N/day)</th>
<th>Fractional synthetic rate&lt;sup&gt;d&lt;/sup&gt; (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protein-free</td>
<td>$-17.5 \pm 4.8^{1e}$</td>
<td>49.6 ± 3.7&lt;sup&gt;1&lt;/sup&gt;</td>
<td>32.1 ± 4.8&lt;sup&gt;1&lt;/sup&gt;</td>
<td>1.34 ± 0.19&lt;sup&gt;1&lt;/sup&gt;</td>
</tr>
<tr>
<td>Low protein</td>
<td>$-3.8 \pm 3.5^{2}$</td>
<td>57.5 ± 6.6&lt;sup&gt;2&lt;/sup&gt;</td>
<td>53.6 ± 3.5&lt;sup&gt;2&lt;/sup&gt;</td>
<td>1.96 ± 0.21&lt;sup&gt;2&lt;/sup&gt;</td>
</tr>
<tr>
<td>Control</td>
<td>$23.3 \pm 8.0^{3}$</td>
<td>91.2 ± 15.6&lt;sup&gt;3&lt;/sup&gt;</td>
<td>114.5 ± 8.0&lt;sup&gt;3&lt;/sup&gt;</td>
<td>3.27 ± 0.30&lt;sup&gt;3&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup> Data from Table 5.
<sup>b</sup> Data from Table 6.
<sup>c</sup> Values calculated as the sum of gain and catabolism.
<sup>d</sup> Synthesis of myofibrillar protein as a percentage of total myofibrillar protein.
<sup>e</sup> Mean ± SD for 5 rats. Means in the same column not sharing a common superscript figure are significantly different ($p < 0.05$).
Table 8. Incorporation of $^{15}$N into myofibrillar protein and exogenous contribution to myofibrillar protein synthesis.

<table>
<thead>
<tr>
<th>Diet</th>
<th>Administered $^{15}$N (mg N)</th>
<th>$^{15}$N incorporated into myofibrillar protein in 24 hr (mg N)</th>
<th>% excess $^{15}$N atom (%)</th>
<th>Recovery rate (%)</th>
<th>Intake N$^b$ (mg N/day)</th>
<th>Exogenous synthesis (mg N/day)</th>
<th>Fractional rate$^d$ (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low protein</td>
<td>9.9±0.3</td>
<td>0.093±0.017</td>
<td>0.073±0.010</td>
<td>28.0±5.4</td>
<td>52.4±1.8</td>
<td>69.7±14.8</td>
<td>0.59±0.011</td>
</tr>
<tr>
<td>Control</td>
<td>10.2±0.1</td>
<td>0.083±0.017</td>
<td>0.073±0.010</td>
<td>25.8±4.4</td>
<td>271.2±12.7</td>
<td>69.7±14.8</td>
<td>1.99±0.42</td>
</tr>
</tbody>
</table>

$^a$ Values calculated by multiplying intake N by $^{15}$N recovery rate.

$^b$ Intake on day 22.

$^c$ Values calculated as a percentage of total myofibrillar protein on day 22 (Table 5).

$^d$ Exogenous synthesis as a percentage of total myofibrillar protein.

Means in the same column not sharing a common superscript figure are significantly different ($p<0.05$).
Table 9. Contribution of endogenous synthesis (total synthesis minus exogenous synthesis) to myofibrillar protein synthesis.

<table>
<thead>
<tr>
<th>Diet</th>
<th>Protein synthesis&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Exogenous synthesis&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Endogenous synthesis&lt;sup&gt;c&lt;/sup&gt;</th>
<th>Endogenous synthesis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(mg N/day)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Protein-free</td>
<td>32.1 ± 4.8&lt;sup&gt;1&lt;/sup&gt;</td>
<td>0&lt;sup&gt;1&lt;/sup&gt;</td>
<td>32.1 ± 4.8&lt;sup&gt;1&lt;/sup&gt;</td>
<td>1.34 ± 0.19&lt;sup&gt;1&lt;/sup&gt;</td>
</tr>
<tr>
<td>Low protein</td>
<td>53.6 ± 3.5&lt;sup&gt;2&lt;/sup&gt;</td>
<td>14.7 ± 3.1&lt;sup&gt;2&lt;/sup&gt;</td>
<td>38.9 ± 3.5&lt;sup&gt;2,3&lt;/sup&gt;</td>
<td>1.43 ± 0.18&lt;sup&gt;1&lt;/sup&gt;</td>
</tr>
<tr>
<td>Control</td>
<td>114.5 ± 8.0&lt;sup&gt;3&lt;/sup&gt;</td>
<td>69.7 ± 14.8&lt;sup&gt;3&lt;/sup&gt;</td>
<td>44.8 ± 8.0&lt;sup&gt;3&lt;/sup&gt;</td>
<td>1.28 ± 0.25&lt;sup&gt;1&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup> Data from Table 7.
<sup>b</sup> Data from Table 8.
<sup>c</sup> Calculated by subtracting the exogenous synthesis from the total synthesis.
<sup>d</sup> Endogenous synthesis as a percentage of the total myofibrillar protein on day 22 (Table 5).
<sup>e</sup> Endogenous synthesis as a percentage of the total protein synthesis.
<sup>f</sup> Endogenous synthesis as a percentage of the amount of catabolized protein (Table 6).
<sup>g</sup> Mean ± SD for 5 rats. Means in the same column not sharing a common superscript figure are significantly different (p < 0.05).
expressed as fractional rates the values were constant. That is, protein intake had no effect on the fractional rate of endogenous synthesis. These results indicate that changes of protein synthesis were affected more by changes of exogenous synthesis than by those of endogenous synthesis.

In the protein deficiency groups, the fractional rates of catabolism and endogenous synthesis were not significantly different from those of the control group, but the ratios of endogenous synthesis to catabolism, that is, the reutilization ratios, were significantly higher. The values were about 65, 68 and 49% in the groups on a protein-free diet, low protein diet and control diet, respectively. Since the absolute amount of catabolism was decreased in protein-deficient groups, the increased reutilization of endogenous N must have resulted in lower contributions of myofibrillar protein to N excretion in these groups.

For comparison of the gains, catabolisms, syntheses and exogenous and endogenous syntheses of myofibrillar protein in the three groups, the fractional rates are summarized in Fig. 2.

**DISCUSSION**

*Rationale of the estimations of the rates of catabolism and exogenous synthesis*

Several methods have been used to estimate the catabolic rate. Most of these are based on the rate of loss of radioactive isotope from tissue proteins, but it is difficult to determine the true catabolic rate in this way, because the radioisotope is reutilized. Millward (9) found that $^{14}$C-glutamate and $^{14}$C-aspartate were reutilized less than the other labelled amino acids, and therefore estimated the rate of catabolism of muscle protein using these amino acids (10). However, the problem of reutilization still remains (11).
The best method for determining myofibrillar protein catabolism seems to be measurement of urinary 3-methylhistidine (1, 2). Although 3-methylhistidine in myofibrils is present only in the actin and myosin fractions (12-14), use of this amino acid for measuring the total myofibrillar protein catabolism seems to be valid because these two fractions together constitute about 80% of the total myofibrillar protein (15) and because the turnover rates of the myofibrillar proteins, actin, myosin and tropomyosin, are similar (16, 17). Some workers have reported slight differences in the turnover rates of these proteins (18, 19), but these differences are not sufficiently great to invalidate the method.

The amount of catabolism was calculated assuming that 80% of the urinary 3-methylhistidine was derived from myofibrils. This assumption is based on a recent report that the contributions of skin and the gastrointestinal tract to 3-methylhistidine excretion amounted to about 17% of the total in normal adult rats (8). Taking this value into consideration with the small amount that is probably derived from tissues other than muscle, skin and the gastrointestinal tract, we assumed that about 20% of the urinary 3-methylhistidine originated from tissues other than muscle.

Protein synthesis can be thought of as two-part, the exogenous and the endogenous, on the basis of the origin of the nitrogen entering into the pool. This idea is based on the theory of the dynamic state of protein metabolism (20) and is not consistent with the theory of the dichotomy of nitrogen metabolism by Folin (21). Naturally, the amino acids derived from the different sources are mixed in the precursor pool for protein synthesis. However, as dietary amino acids can be traced by labelled amino acid, protein synthesized with those (exogenous synthesis) can be determined quantitatively but not qualitatively. The endogenous synthesis also can be estimated from the exogenous and total syntheses. In this study exogenous synthesis was calculated from the recovery of isotope in myofibrillar protein 24 hr after oral administration of $^{15}$N-leucine. The first point in considering the validity of this method is whether the recovery of isotope 24 hr after administration of $^{15}$N-leucine actually represents the utilization of exogenous N. $^{15}$N-Labelled amino acid can be thought to represent exogenous N only until it is incorporated into tissue protein; its movement thereafter is an indication of tissue protein metabolism. Friedberg et al. (22) studied the time course of incorporation of $^{35}$S-methionine administered intravenously. They found that the specific activity in most tissues reached a peak 3 to 16 hr after its injection and then remained fairly constant for at least 24 hr. Similar results have been obtained in our laboratory (23). A second point about the method is whether $^{15}$N-leucine can be assumed to represent whole dietary amino acids. This seems a valid assumption because, when various essential amino acids labelled with $^{14}$C were administered to rats, the recovery rates of radioactivity in various tissues were not affected by the type of amino acid used (24).
Effects of diets on the rates of synthesis, catabolism and reutilization

Gain of tissue protein is a result of the difference between protein synthesis and catabolism. In this study, rats lost more myofibrillar protein when protein intake was restricted, because the catabolic rate exceeded the synthetic rate (Tables 6 and 7; Fig. 2). However, the catabolic rates in the protein restriction groups were not significantly different from that of the control group, suggesting that gain (or loss) of myofibrillar protein may depend on changes in the synthesis rate rather than the catabolic rate. Some workers have reported that restriction of protein intake suppressed the catabolic rate of muscle protein (25–28), while others have reported the reverse (10, 29). These contradictory results may be due to differences in methodology, sampling procedures (whole muscle or fractionated muscle) or experimental design including dietary conditions and the species, age and sex of the animals used. However, the results all appear to show that the rate of protein synthesis is altered more than that of catabolism by differences in dietary conditions. The finding that the catabolic rate is not suppressed in protein restriction seems inappropriate for preventing protein loss. However, it may be homeostatically desirable, since breakdown of muscle protein can contribute to maintenance of circulating amino acids and glucose (30). This conclusion is supported by the following observations. In protein-deficient infants, the level of protease is usually the same as that in normal infants. However, when these protein-deficient infants contract an infectious disease, the level of protease inhibitor increases and as a result the catabolic rate of muscle protein is decreased. This reduces the supply of amino acids and energy to maintain homeostasis and symptoms of malnutrition, such as marasmus and kwashiorkor, appear (31). Accordingly, the fact that the rate of catabolism of muscle protein does not alter under different dietary conditions seems to be favorable for homeostasis during protein restriction.

We found that the rate of synthesis of myofibrillar protein increased with increase in protein intake (Table 7, Fig. 2). This is in good agreement with results from other workers who estimated the values using 14C-labelled amino acids (25, 29, 32). There are also several reports showing that the increased rate of synthesis is due to increased ribosome activity or RNA concentration (33, 34). There are no previous reports on the measurements of exogenous and endogenous syntheses of myofibrillar protein (Tables 8 and 9; Fig. 2). We found that the fractional rates of endogenous synthesis were similar in the three groups (Table 9), but that the rate of exogenous synthesis was proportional to the protein intake (Table 8). These observations indicate that exogenous synthesis regulates the rates of synthesis and gain of myofibrillar protein.

It has been suggested that the extent of amino acid reutilization increases in protein deficiency (35), but there have been few quantitative studies on this phenomenon. Amino acid reutilization in muscle was estimated as 10–30% in the studies of Gan and Jeffay (36) and Waterlow and Stephen (37). On the other hand, we appraised the reutilization rate to be about 50% on the control diet. Such a
great difference in the rates must be due to the difference in the interpretation for reutilization. There are two interpretations; one is the reutilization of amino acids within the same tissue and the other is the reutilization of those derived not only from self tissue but also from other tissues. The estimation reported by Gan and Jeffay and Waterlow and Stephen belongs to the former interpretation and that of our study belongs to the latter. Naturally, the rates of the latter type are higher than that of the former type. Considering the review of Garlick et al. (35) that under normal dietary conditions about 80% of amino acids liberated by protein breakdown are reused for synthesis in a whole body, our result may be considered not to be too high.

In the groups on protein-free and low protein diets, the reutilization rate was about 70%. This increased reutilization during protein restriction seems to very favorable for preventing loss of nitrogen from the body.

We wish to thank Mr. T. Korin and Miss. N. Uezu for their technical assistance during this investigation.

REFERENCES


