Body Composition and Utilization of Protein and Energy in Growing Rats at Different Dietary Protein to Energy Ratios by Use of Purified Whole Egg Protein

Hideyuki TANAKA, Michio YAMAGUCHI and Masao KAMETAKA

Department of Animal Sciences and Agricultural Chemistry, Faculty of Agriculture, The University of Tokyo, Tokyo

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A study has been made on growing rats to investigate the effect of variation in percentage of dietary protein calories from 0 to 50% by the use of purified whole egg protein on the growth, food efficiency, protein efficiency ratio (PER), body composition, and efficiencies of protein and energy utilization.

Body weight gain and PER attained a maximum, and food efficiency reached a plateau at 10 PC% (protein calories percent) in the diet, having a constant metabolizable energy content (410 kcal). Body and liver compositions changed in systematic patterns, where liver lipid content showed a specific increase at 5 PC%.

Body protein retention reached a plateau at 15 PC% but with little difference from the value at 10 PC%, while body lipid retention give a maximum at 10 PC% showing a gradual decrease thereafter.

Throughout the given dietary protein to energy ratios, energy utilization was constant when expressed as the increment of body energy retention divided by the increment of metabolizable energy intake. At and above 12 or 13 PC%, the efficiency of net body protein energy retention against metabolizable energy intake was constant at about 12.5% on the average.

It has long been recognized that the efficiency of protein utilization of the diet is affected not only by the quality and quantity of protein itself, but also by the energy content of the diet. The growth, efficiency of protein and body composition of the animal have been widely examined with reference to the dietary energy and protein levels, a part of which was reviewed by Munro as the protein sparing action. In the field of poultry nutrition, the calorie to protein ratio of diets has been recognized as an important factor for the productivity of eggs and meat. Since Miller and Payne presented a net dietary protein caloreis percent (NDpCal %) as a useful index for dietary protein evaluation, the importance of the caloric representation of retention efficiency of dietary protein has also been recognized in human nutrition.

The protein sources used in these studies have not, in most cases, been whole egg protein, and in the case of whole egg protein its purity has been 70 to 80%. Therefore, the inappropriate factors such as limiting amino acid in proteins other than whole egg protein and the energy of impurities, mostly fat, of the whole egg protein have not been eliminated.

This study aims at investigating the metabolic fate of proteins or individual amino acid in the bodies of growing rats in relation to the balance data on the dietary protein and energy metabolism at various dietary protein to energy ratios using purified whole egg protein. In this paper the data on the growth, efficiencies of dietary protein and energy utilization, and body composition are presented, serving as a guide to dietary conditions for further studies on the metabolism of the carbon skeletons of individual amino acids, which will be published subsequently.
**EXPERIMENTAL**

**Animals and diets.** Male weanling Wistar rats were preliminarily fed a stock diet (CLEA CE-II, crude protein: 24.0%) until the mean body weight reached about 70 g. The animals were then divided into 9 groups of 6 animals each. One group was immediately killed to determine the initial body composition. The other 8 groups were thereafter given the experimental diets with graded levels of protein calories percent at 410 kcal of total metabolizable energy (0, 5, 10, 15, 20, 25, 30, 50 PC%) for three weeks.

The composition of the experimental diets is shown in Table I. The protein calories percent (PC%) means the proportion of metabolizable energy of dietary protein to the total metabolizable energy of the diet. The metabolizable energy of dietary ingredients was taken from the Atwater factors, i.e., 4.0 kcal per g for whole egg protein and corn starch, 9.0 for lard, and 0.0 for cellulose powder. In this experiment, the protein calories percent of the diets were similar to the weight percent of dietary protein owing to the inclusion of 10% lard and 5% cellulose powder. The gross energy (heat of combustion) of experimental diets in Table I was calculated from the energy values of dietary ingredients shown in Table II. The gross energy values of the dietary ingredients and body components are shown in Table II. The animals were maintained in individual cages with free access to food and water. The room temperature was kept constant at 22°C. Body weight and food consumption were recorded on alternate days during the experimental period.

**Analyses.** After the animal was killed by anesthetizing with chloroform, the intact liver was removed and the gastrointestinal contents were discarded by washing with water. The liver and carcass (the remainder) were weighed and stored in a deep-freezer maintained at −20°C for later analyses. The liver was minced into small pieces as much as possible with scissors. The carcass was cut into several pieces with a chopper and then minced 4 to 5 times with a meat grinder until the sample appeared to be homogeneous. The weights of the minced samples of liver and carcass taken for analyses were approximately 1 and 4 g for moisture and ash, 0.3 and 0.6 g for protein, and 2 and 20 g for lipid, respectively. The moisture content was determined by drying the samples at 105°C, and the ash determination was made in succession by ashing the dried samples at 560°C. Nitrogen was analyzed by the semi-micro Kjeldahl method, and lipid by Folch's method in which the extraction with a chloroform-methanol mixture was conducted using a Potter-Elvehjem homogenizer for the liver and a blender for the carcass. The energies of body lipid and body protein were determined on the extract and residue fractions of Folch's method by the bomb calorimeter mentioned above. The gross energy values of the dietary ingredients and body components are shown in Table II.
RESULTS

As shown in Fig. 1, the growth curves of the rats were almost linear in groups of more than 15 PC%, while those of the 5 and 10 PC% groups were slightly retarded for about the first week of the experimental period.

Figure 2 shows the body weight gain, food efficiency (body weight gain/food intake) and protein efficiency ratio (PER) for the entire period of the experiment. Body weight gain reached a maximum of 7.3 g per day in the 10 PC% group, followed by the decrease in the higher PC% groups. Even the 5 PC% group gained about 2 g per day, apparently reflecting the good quality of whole egg protein. Food efficiency attained a complete plateau of 0.4 in the 10 PC% group. PER showed a regular pattern as has generally been observed, with a maximum of 3.9 in the 10 PC% group. The above three values obtained were higher than those which had been observed previously in this type of study.

The overall data such as protein and energy intake, and their retentions are summarized in Table III. The values of protein and energy

![Fig. 1. Growth Curves of Rats Fed Diets with Various Protein to Energy Ratios for Experimental Period of 3 Weeks. A: 0, B: 5, C: 10, D: 15, E: 20, F: 25, G: 30, H: 50 PC%.

![Fig. 2. Effect of Various Dietary Protein to Energy Ratios on Body Weight Gain, Food Efficiency and Protein Efficiency Ratio. ○ body weight gain, ■ food efficiency, ● protein efficiency ratio. (PER)

TABLE III. FOOD, PROTEIN AND ENERGY INTAKES, PROTEIN AND ENERGY RETentions AND EFFICIENCY OF NET BODY PROTEIN RETention OF RATS DURING THE THREE WEEK EXPERIMENTAL PERIOD

<table>
<thead>
<tr>
<th>Diets (Protein calories percent)</th>
<th>0</th>
<th>5</th>
<th>10</th>
<th>15</th>
<th>20</th>
<th>25</th>
<th>30</th>
<th>50</th>
</tr>
</thead>
<tbody>
<tr>
<td>Food intake (g)</td>
<td>109a</td>
<td>236</td>
<td>364</td>
<td>342</td>
<td>336</td>
<td>324</td>
<td>301</td>
<td>300</td>
</tr>
<tr>
<td>Protein intake (g)</td>
<td>0.0</td>
<td>12.1</td>
<td>37.4</td>
<td>52.6</td>
<td>68.8</td>
<td>83.1</td>
<td>92.4</td>
<td>154.0</td>
</tr>
<tr>
<td>Metabolizable energy intake (kcal)</td>
<td>449</td>
<td>967</td>
<td>1490</td>
<td>1400</td>
<td>1380</td>
<td>1330</td>
<td>1230</td>
<td>1230</td>
</tr>
<tr>
<td>Body energyb retention (kcal)</td>
<td>-18.6</td>
<td>195</td>
<td>488</td>
<td>441</td>
<td>446</td>
<td>381</td>
<td>399</td>
<td>340</td>
</tr>
<tr>
<td>Body proteinc retention (g)</td>
<td>-2.3</td>
<td>7.0</td>
<td>24.8</td>
<td>26.9</td>
<td>25.7</td>
<td>26.2</td>
<td>25.2</td>
<td>26.5</td>
</tr>
<tr>
<td>Efficiency of net body protein retention (%)</td>
<td>76.9</td>
<td>72.5</td>
<td>55.5</td>
<td>40.7</td>
<td>34.3</td>
<td>29.8</td>
<td>18.7</td>
<td></td>
</tr>
</tbody>
</table>

a Mean values for six rats.

b Final body energy — Initial body energy; body energy is calculated from Table II.
c Final body protein — Initial body protein.
Carcass and liver compositions are presented in Tables IV and V, respectively. As for carcass composition, the moisture content of the 10 PC% group was reduced to the greatest degree accompanied by the largest increase in lipid content. In general, the increase or decrease of moisture content was associated with the decrease or increase of lipid content. Although the lipid content of the 0 PC% group remained at the initial value, it rose remarkably in the 5 PC% group and gave a

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**TABLE IV. CARCASS COMPOSITION OF RATS RECEIVING DIETS WITH VARIOUS PROTEIN TO ENERGY RATIOS**

<table>
<thead>
<tr>
<th>Protein calories percent</th>
<th>Moisture (%)</th>
<th>Protein (%)</th>
<th>Lipids (%)</th>
<th>Ash (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Initial)</td>
<td>72.4±0.8</td>
<td>16.7±0.5</td>
<td>6.7±0.4</td>
<td>2.9±0.2</td>
</tr>
<tr>
<td>0</td>
<td>69.8±1.5</td>
<td>18.4±0.5</td>
<td>8.2±1.0</td>
<td>5.0±1.0</td>
</tr>
<tr>
<td>5</td>
<td>61.0±2.5</td>
<td>16.1±1.0</td>
<td>19.7±3.7</td>
<td>3.1±0.4</td>
</tr>
<tr>
<td>10</td>
<td>58.8±1.3</td>
<td>16.7±0.3</td>
<td>21.4±1.0</td>
<td>2.3±0.2</td>
</tr>
<tr>
<td>15</td>
<td>61.4±1.6</td>
<td>17.9±0.7</td>
<td>18.0±2.1</td>
<td>2.7±0.4</td>
</tr>
<tr>
<td>20</td>
<td>60.5±3.2</td>
<td>17.7±0.7</td>
<td>19.0±4.3</td>
<td>2.6±0.3</td>
</tr>
<tr>
<td>25</td>
<td>63.5±2.3</td>
<td>18.1±0.8</td>
<td>15.4±2.3</td>
<td>2.4±0.2</td>
</tr>
<tr>
<td>30</td>
<td>62.1±2.4</td>
<td>18.1±0.5</td>
<td>17.2±2.6</td>
<td>2.3±0.5</td>
</tr>
<tr>
<td>50</td>
<td>64.4±0.5</td>
<td>18.8±0.8</td>
<td>13.7±1.0</td>
<td>2.6±0.2</td>
</tr>
</tbody>
</table>

*Mean values with standard deviations for groups of six rats.*

**TABLE V. LIVER COMPOSITION OF RATS RECEIVING DIETS WITH VARIOUS PROTEIN TO ENERGY RATIOS**

<table>
<thead>
<tr>
<th>Protein calories percent</th>
<th>Moisture (%)</th>
<th>Protein (%)</th>
<th>Lipids (%)</th>
<th>Ash (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Initial)</td>
<td>74.1±1.5</td>
<td>21.2±0.7</td>
<td>4.3±0.2</td>
<td>1.3±0.1</td>
</tr>
<tr>
<td>0</td>
<td>76.9±0.7</td>
<td>15.8±0.6</td>
<td>4.2±1.1</td>
<td>1.2±0.1</td>
</tr>
<tr>
<td>5</td>
<td>74.3±0.6</td>
<td>16.1±1.0</td>
<td>6.8±0.8</td>
<td>1.2±0.1</td>
</tr>
<tr>
<td>10</td>
<td>74.8±0.6</td>
<td>16.9±0.3</td>
<td>4.7±0.8</td>
<td>1.3±0.2</td>
</tr>
<tr>
<td>15</td>
<td>74.7±1.3</td>
<td>17.9±0.7</td>
<td>4.9±0.9</td>
<td>1.2±0.1</td>
</tr>
<tr>
<td>20</td>
<td>74.7±0.8</td>
<td>18.5±1.1</td>
<td>4.4±0.4</td>
<td>1.2±0.03</td>
</tr>
<tr>
<td>25</td>
<td>75.0±0.5</td>
<td>19.1±0.3</td>
<td>4.3±0.5</td>
<td>1.2±0.02</td>
</tr>
<tr>
<td>30</td>
<td>74.9±0.4</td>
<td>18.9±0.9</td>
<td>4.3±0.3</td>
<td>1.2±0.03</td>
</tr>
<tr>
<td>50</td>
<td>73.7±0.5</td>
<td>20.1±0.5</td>
<td>4.6±0.6</td>
<td>1.2±0.04</td>
</tr>
</tbody>
</table>

*Mean values with standard deviations for groups of six rats.*

retention were determined as the difference between the body protein and body energy of animals given the experimental diets and those of animals killed at the start of the experiment, *i.e.*, 10.6 g and 98.2 kcal, respectively. Both food intake and metabolizable energy intake reached a maximum in the 10 PC% group, with a gradual decrease in the higher PC% groups. On the other hand, protein intake increased in an approximate proportion to the dietary protein level. Body energy retention was raised markedly in the 0 to 10 PC% groups, with maximum value in the 10 PC% group. A gradual decrease of the retention was observed in the higher PC% groups.

Body protein retention was raised in a similar manner to body energy retention but to a slightly less degree, with a maximum value in the 15 PC% group but with little difference from the value of 10 PC% group. The protein retention, however, was maintained at an almost constant value in the higher PC% groups. The efficiency of net body protein retention showed high values in the 5 and 10 PC% groups and then decreased with a similar pattern to that usually observed for NPU (net protein utilization).27)
Protein and Energy Utilization in Growing Rats

FIG. 3. Effect of Various Dietary Protein to Energy Ratios on Retention of Body Protein and Body Lipid over Experimental Period of 3 Weeks.

- - body protein, ○○ body lipid.

blunt peak in the 5 to 20 PC% groups. The increased contents of protein and ash in the 0 PC% group were probably due to the reduction of lipid content. The protein content showed a gradual but slight increase as the dietary protein level increased. The ash content remained constant, except in the 0 PC% groups as described above.

As for liver composition, the lipid content of 5 PC% group was significantly increased to a level of the so-called fatty liver (P<0.05), with a single sharp peak in marked contrast to the case of carcass lipid. The protein content increased gradually as the dietary protein level increased. The moisture and ash contents were almost unchanged over the whole range of dietary groups.

The retention of body protein and body lipid for the entire period of the experiment is shown in Fig. 3. Body protein retention increased almost curvilinearly with increasing levels of dietary protein calories percent until a plateau was attained between the 10 and 15 PC% groups. It should be noted that a constant retention of body protein (see also Table III) was observed in a wide range from 15 to 50 PC% where the body weight gain tended to decline.

Body lipid retention, which clearly exceeded the protein retention in a range of 5 to 20 PC% also increased curvilinearly until a maximum value was obtained in the 10 PC% group, and thereafter decreased to a marked degree intersecting with the protein retention at around 30 PC%. It is of considerable interest that the greatest excess in retention of body lipid over that of body protein was observed in the 10 PC% group where the maximum body weight gain was obtained.

The proportions of retained energy in terms of whole body, body lipid, and body protein to the metabolizable energy intake are each plotted against the dietary protein calories percent in Fig. 4. The efficiency of energy retention for the whole body energy reached a maximum at about 30% in the 10 PC% group, and thereafter decreased gradually but with little difference from the values for the 10 and 50 PC% groups. The energy retention of body lipid showed a similar pattern to that of the whole body. In other words, the pattern of body energy retention was naturally reflected by that of body lipid because of its greater content and high energy value.

On the other hand, the proportion of energy retention as body protein showed a linear increase in a range from 0 to 10 PC% and attained a plateau at a value about 11% between the 10 and 15 PC% groups. It should be noted that the animals retained a constant amount of body protein corresponding to 11%
of the dietary metabolizable energy consumed in a wide range from 15 to 50 PC%. Almost the same pattern was obtained when the body protein energy retention expressed as the numerator was replaced by the net body protein energy retention of each protein-fed group. The value of the plateau in this case was shown to be about 12.5%. This value was in good agreement with 12 to 13 dietary protein calories percent where the plateau was obtained. This fact implies that about 12.5 PC% in the diet of this size is most preferred to secure the maximum efficiency of protein retention in the body and that whole egg protein makes it possible owing to its adequate amino acid pattern.

Although the whole body energy retention showed an initial increase up to 10 PC% and a subsequent gradual decrease with increasing levels of dietary protein calories percent as described above, a good linear fit was obtained when the body energy retention was plotted against the metabolizable energy intake as shown in Fig. 5. The regression equation describing this relationship was found to be $Y = -251 + 0.495 \times (r=0.989)$, where $Y$ is body energy retention (kcal) and $X$ is metabolizable energy intake (kcal). From this equation it is suggested that about 50% of the increment of metabolizable energy intake was retained as body energy of the animal irrespective of dietary protein calories percent. This efficiency naturally means the net efficiency of energy retention which was needed for maintenance as well as for growth of the animals.

DISCUSSION

By using purified whole egg protein as a dietary protein source, it was possible to study the effects of different protein to energy ratios of the diet on the protein metabolism without inappropriate factors such as limiting amino acids and the usual concomitant impurities such as lipids of egg yolk.

In the present study, a maximal growth was obtained in rats fed the diet containing 10 PC% (equiv. to 11.2 weight %) of whole egg protein. This result agreed well with that of Mitchell et al., who reported that a maximal growth was obtained at 12 or more weight % of dietary protein, but using unpurified whole egg protein.

However, a maximal retention of body protein was attained in the 15 PC% group but with little difference from the value for the 10 PC% group. With the data of body lipid retention, it was clearly shown that the growth promotion at 10 PC% of the diet was attributed mainly to the accelerated accumulation of body lipid. Although the protein requirements for growth must be considered extensively in relation to other physiological data such as the so-called protein reserves, it was suggested that about 12 to 13 PC% of dietary protein is required for maximal growth provided that sufficient amounts of essential amino acids are supplied. The growth retardation at the initial stage in the 5 and 10 PC% groups may be due to the metabolic adaptation to lower protein intake.

The compositions of carcass and liver differed with the different protein to energy ratios of diets. The relationship between the carcass and liver lipids seemed to have a metabolic importance, e.g., for fat accumulation generally
observed in the low-protein and in amino acid imbalanced diets and also for obesity. Carcass lipid gave a blunt peak of accumulation over a wide range of 5 to 20 PC% in the diets, while liver lipid showed a considerably high accumulation in the 5 PC% diet only. A similar relationship between the carcass and liver lipids has been observed by Velu et al.\textsuperscript{16)} with chicks using balanced amino acid mixtures. The additional data on the lipid accumulation of liver at 2.5 and 7.5 PC% in the diets, which were obtained as a part of this study, showed that the lipid accumulation in the liver was characteristic at 5 or a little more PC% in the diet. The lipid accumulation in liver of the 5 PC% group is considered to have occurred to the level of the so-called fatty liver.\textsuperscript{20)} Many studies have been performed on the accumulation of liver lipid in growing rats fed low protein diets,\textsuperscript{20,24,25)} but these experimental results differed considerably probably due to the different dietary proteins and energy sources as well as the different intake levels.

The present results on liver lipid accumulation may also be associated with the fatty liver induced by an amino acid imbalanced diet of 8% casein plus 0.3% methionine, since the levels of utilisable essential amino acids in the imbalanced diet are considered to be similar to those in the 5 PC% (5.6 weight %) whole egg protein diet in this study. Although the mechanism of fat accumulation in the liver in the low protein diets and the amino acid imbalanced diet has not been sufficiently clarified,\textsuperscript{21~26)} it can be said that the protein to energy ratio of the diet is an important factor in causing fat accumulation in the liver. This accumulation might also be closely associated with the body fat accumulation, which will be discussed in a subsequent paper.

The body lipid retention in the protein-free diet declined to $-0.6 \text{ g}/3 \text{ weeks}$ in spite of sufficient supply of dietary non-protein energy. For the 5 PC% diet, it was stimulated to the level of more than twice the body protein retention (Fig. 3), and occupied the major part of body weight gain, 2 g/day (Fig. 2). For the 10 PC% diet, the lipid retention reached a maximum, but with the smaller ratio to the protein retention than for the 5 PC% diet. On the other hand, diets of 15 or higher PC% reduced the body lipid retention probably due to the depression of body fat synthesis by the decreased supply of non-protein energy in diets and also due to the reduction in food consumption. These results suggest that the body lipid retention could be accounted for by a mathematical function of dietary protein intake.

As shown in Fig. 4, the efficiency of energy utilization varied with the different levels of dietary protein calories percent. When plotted against the metabolizable energy intake, a linear relationship was obtained in the whole range of dietary protein calories percent. In other words, the increment of energy retention divided by the increment of metabolizable energy intake was constant irrespective of the various dietary protein to energy ratios. A similar relationship was obtained for protein and lipid retention plotted against dietary protein intake in the range of 0 to 10 PC% in diets. These linear relationships have also been presented by Velu et al.\textsuperscript{16)}

The estimation of the efficiency of net energy retention is considered to be significant, since part of the metabolizable energy of dietary components is naturally used for the maintenance of body components. As for the efficiency of net body protein energy retention, a meaningful value of about 12.5% was obtained on the plateau ranging from the 15 to 50 PC% groups. The efficiency of net body energy retention, however, was estimated to be about 50% over the whole range of dietary protein calories percent from the slope of the regression line as shown in Fig. 5.

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