EFFECTS OF DIETARY PROTEIN LEVEL ON THE ENERGY METABOLISM OF RATS DURING EXERCISE

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Summary The experiments were designed to study the effects of exercise on various intensities in albino rats fed diets containing 8 per cent or 20 per cent casein. Male rats about 7 weeks old were divided into exercise groups and control (non-exercise) groups. The exercise groups were forced to run 57 km or 76 km in total during a 28-day period. The oxygen uptake of rats was measured in order to study the influence of exercise on energy metabolism of rats and the effects of training. Body weight and food intake were also measured.

1. In all exercise groups the total amount of food intake and body weight gain during the exercise period were smaller than those of the control groups, regardless of the protein levels of the diets. But while the rats were kept at rest after the exercise period, the food intake and body weight of these groups increased and approached those of the control groups.

2. No difference in resting metabolism was observed both in the 8 per cent and 20 per cent casein groups. At lower speeds up to 1.5 km/hr the 8 per cent casein group indicated lower oxygen requirement during the exercise but at speeds higher than 2.0 km/hr the oxygen requirement was definitely higher than that of the 20 per cent casein group. The estimated optimum speed for rats weighing 300 g on the average is somewhere between 1.5 and 2.0 km/hr if calculated on the basis of oxygen requirement for running 100 m.

3. The obvious effects of training on oxygen uptake were seen in the 20 per cent casein group after four weeks' training, although no effect was found in the 8 per cent casein group.
It is generally believed that adequate exercise is favorable for the development of physical fitness in human beings but that over-exercise may have retarding effect. Physiques seem to depend largely upon nutrition during the course of growth and the improved physiques of youth today is generally attributed to the increased intake of protein of better quality during the post-war period, notably since 1955. Physical fitness of the post-war generation, however, is regarded as disproportionate to their improved physiques (1–4).

It is not known exactly how much exercise is necessary for growing youth. Moreover what nutritional conditions are adequate for the maintenance of health and improvement of physical performance proportional to physique are still not clear. It is important to establish answers to these questions for the benefit of nutritional science and sports medicine.

On the other hand, it has been assumed that adequate exercise imposed on experimental animals in their growing stage increased their food intake and hence resulted in increased body weight. But HEARN and WAINIO (5), YAMAOKA et al. (6) and CHRISTENSEN and CRAMPTON (7) found that increase in body weight of rats was discouraged by the exercise.

In order to ascertain the above experimental facts, the authors conducted various series of experiments on male rats in their growing stage. The results were presented elsewhere (8–12). In these experiments male rats subjected to exercise, showed a reduced food intake, which resulted in a lowering of body weight of these rats. The body fat content of these rats, furthermore, decreased significantly. However, the data obtained from these experiments did not sufficiently ascertain the degree of metabolic change in rats caused by exercise, nor did they clarify the relation between the running speed and energy metabolism of each rat.

The experiments in this paper were designed to study the effects of exercise of various grades on the energy metabolism of the rats fed diets containing 8 per cent or 20 per cent casein.

MATERIALS AND METHODS

1) Experimental diets and methods of feeding. Table 1 shows the composition of the experimental diets. The casein (Tanabe Amino-acid Research Foundation) was used as a protein source. The vitamin mixture contained twice the amount described by HARPER (13), which was expected to keep out of the lack in vitamins caused by exercise. The fuel values were 3.48 kcal/g for 8 per cent and 3.55 kcal/g for the 20 per cent casein diet. The animals were fed the diets and water ad libitum.

2) Experimental design. Male rats about 7 weeks old of Sprague-Dawley, JCL strain, weighing 250 g on the average were used for the experiments. Three days of pre-training was given to the rats in order to accustom them to running
Table 1. Composition of experimental diets (%).

| Ingredients               | 8% casein diet | 20% casein diet |
|---------------------------|----------------|-----------------|
| Casein                    | 8              | 20              |
| Corn oil                  | 5              | 5               |
| Salt mixture              | 5              | 5               |
| Vitamin mixture*          | 0.5<sup>b</sup> | 0.5<sup>b</sup> |
| Choline-chloride          | 0.2            | 0.2             |
| Corn starch               | 81.3           | 69.3            |

Vitamin A 15,000 I.U./1,000 g in diet
Vitamin D 1,500 I.U./1,000 g in diet

<sup>a</sup> Contents (mg) per 10 g of vitamin mixture: thiamine, 5.9; riboflavin, 5.9; nicotinamide, 29.4; pyridoxine·HCl, 2.9; folic acid, 0.2; calcium pantothenate, 23.5; vitamin B<sub>12</sub>, 0.2; vitamin K<sub>2</sub>, 0.6; biotin, 0.1; inositol, 117.6; ascorbic acid, 58.8; lactose, 9.755 g.

<sup>b</sup> According to Harper (19). For the other condition see text.

conditions. They were forced to run for 15 min a day at about 1 km/hr on a treadmill made for rats (8). Rats that did not run well in this preliminary running practice were not used in subsequent experiments. The diet given during the pre-training sessions was commercially available rat diet pellets (CE-2 produced by Nihon Clea Inc.). At the end of the preliminary training, the rats were divided into 6 groups as shown in Fig. 1. Each group contained some 5 to 6 animals. Consequently, the average body weight in each group was roughly the same.

As shown in Fig. 1, the rats in the low speed running groups (I and II) were forced to exercise by the following schedule: for the first 2 days, 60 min/day at 1.2 km/hr; from day 3, 60 min/day 1.5 km/hr for 2 days and so on. The high-speed running groups (I') were given the same amount of exercise as the low speed groups for the first 6 days. From the 7th day an increasing amount of exercise was given to those groups in Fig. 1. The exercise rats in group I were trained to run about 57 km in total during the 28-day period. The exercise group in group I' ran about 76 km in total in the same period and non-exercise groups were taken as controls. The exercise rats of group II were given the same amount of exercise as those of group I, but after the exercise period, they were rested for another 12 days. This group was used for measuring the effects of training on the oxygen uptake during exercise and also for measuring the change of food intake and body weight gain during recovery period.

3) Sampling and analysis of expiratory gas. After the 7th day of training, the exercised rats in group I and I' were made to run an additional 0.5, 0.8, 1.0, 1.2, 1.5, 2.0, or 2.5 km/hr for 30 min on a specially designed treadmill for energy metabolism (14), after 15 hr starvation. The control groups were also starved in the same time. These experiments were started from 1 p.m. every three or four days.
The expiratory gas of these rats during running was collected and was analyzed by a thermal conductivity analyzer (Fukuda Breath Analyzer) for oxygen and carbon dioxide. The energy metabolism at rest was also measured prior to each exercise.

RESULTS

As shown in Fig. 2, the food intake of the running groups during exercise was smaller than that of control in each pair. Especially, in group I of the 8 per cent casein diet group, 425 g of the total food intake of the exercise group was much smaller than the 537 g of the control ($P<0.01$) and in groups I and I' of the 20 per cent casein diet groups, 474 g and 522 g of the total food intake of the exercise groups, respectively, were also significantly smaller than those of the controls (546 g and 604 g) ($P<0.05$). While, the value of total food intake divided by final body weight was compared between these two groups. The difference was not significant. After the exercise period, there was a slight increase
in the food intake of the exercise group which was fed the 8 per cent casein diet. The exercise group fed the 20 per cent casein diet showed an increased food intake from the 4th day of the rest period and on the 6th day their food intake was almost the same as that of the control.

Figure 3 shows the average gain in body weight. The groups fed the 20 per cent casein diet gained more than those fed the 8 per cent casein diet. The exercise groups, regardless of intensity of the exercise given and of the protein level in the diet taken, gained less than did the controls. In the rest period after exercise, on the other hand, the body weight gain of the exercise groups caught up gradually with that of the control groups and after the 6th day of the rest period, both groups showed almost same body weight gain.

The process of the body weight gain of the control groups fed the 8 per cent casein diet was almost the same as that of the exercise groups fed the 20 per cent casein diet throughout the 28 days.

The oxygen uptake at rest and oxygen requirement during exercise is shown in Fig. 4. According to the paper of HEGSTED and HAFFENREFFER (13), the basal metabolism varies with the 0.35 power of the body weight in adult rats. The authors calculated all results with 0.35 power of the body weight (G) instead of
Fig. 3. Body weight gain. The curves show body weight gain of different groups, measured every two days. Symbols denote the groups: ●, exercise (I); ○, control (I); ▼, exercise (I'); ◄, control (I'); ▲, exercise (II); △, control (II).

with the body weight for energy metabolism. No difference in the resting metabolism of rats was observed both in the 8 per cent and 20 per cent casein diet groups. Oxygen requirements during exercise were obtained by subtracting the respectively applicable metabolic value at rest from each oxygen uptake for each speed. Oxygen requirement during exercise was larger as the running speeds were faster. The tendency was clearer over 1.5 km/hr for 8 per cent casein diet group and over 2.0 km/hr for 20 per cent casein group. Oxygen requirement during exercise was significantly lower (P<0.05) in the 8 per cent casein group than in the 20 per cent casein group at a low speed (0.5 km/hr), but at a higher speed (2.0 km/hr and more) it seemed to be lower in the 20 per cent casein group than in the 8 per cent casein group.

Table 2 shows carbon dioxide excretion at rest and during exercise for 30 min. Net carbon dioxide excretion due to exercise as obtained by subtracting carbon dioxide excretion at rest from that during exercise increased as the speed became faster.

Table 3 shows respiratory quotient (R.Q.) during exercise at different speeds. The values of the 8 per cent casein group at lower speeds were all slightly higher than of the 20 per cent casein group and at higher speeds the 20 per cent casein
Fig. 4. Oxygen uptake at rest and oxygen requirement during exercise for 30 min. Symbols denote: ●, O₂ uptake at rest (8%); ▲, O₂ uptake at rest (20%); ○, O₂ requirement during exercise (8%); △, O₂ requirement during exercise (20%). Bars denote confidence limits (P<0.05).

Table 2. Carbon dioxide excretion at rest and during exercise for 30 min.*

| Prot. level | 0.5 | 0.8 | 1.0 | 1.2 | 1.5 | 2.0 | 2.5 |
|-------------|-----|-----|-----|-----|-----|-----|-----|
| CO₂ excretion at resting metabolism (ml/G³/min) | 0.57 ± 0.12 | 0.56 ± 0.10 | 0.60 ± 0.18 | 0.59 ± 0.14 | 0.57 ± 0.05 | 0.58 ± 0.05 | 0.62 ± 0.14 |
| At running—at rest (ml/G/min) | 0.43 ± 0.19 | 0.67 ± 0.25 | 0.65 ± 0.23 | 0.73 ± 0.19 | 0.76 ± 0.10 | 1.23 ± 0.37 | 1.56 ± 0.18 |
| CO₂ excretion at resting metabolism (ml/G³/min) | 0.50 ± 0.06 | 0.47 ± 0.07 | 0.50 ± 0.05 | 0.49 ± 0.05 | 0.48 ± 0.08 | 0.51 ± 0.10 | 0.56 ± 0.10 |
| At running—at rest (ml/G/min) | 0.51 ± 0.14 | 0.66 ± 0.13 | 0.69 ± 0.26 | 0.82 ± 0.21 | 0.88 ± 0.16 | 1.12 ± 0.28 | 1.57 ± 0.39 |

* Values are mean ± half range of confidence interval (confidence limits) (P<0.05).

³ G means 0.35 power of the body weight.
Table 3. Respiratory quotient during exercise at different speeds. The ratio between oxygen requirement during exercise and carbon dioxide excretion due to exercise was calculated as the R.Q.

| Prot. level | 0.5  | 0.8  | 0.74  | 0.83  | 0.75  | 0.84  | 0.85  |
|-------------|------|------|-------|-------|-------|-------|-------|
| 8%          | ±0.13| ±0.20| ±0.08 | ±0.09 | ±0.08 | ±0.07 | ±0.09 |
| 20%         | ±0.08| ±0.06| ±0.07 | ±0.06 | ±0.07 | ±0.16 | ±0.09 |

*Values are confidence limits (P<0.05).

Fig. 5. Oxygen requirement for a 100 m running at different speeds. Symbols denote: ○, 8% casein diet group; △, 20% casein diet group. Bars denote confidence limits (P<0.05).

The group showed higher values than did the 8 per cent casein group. But the results did not show any significant difference between the 8 per cent and 20 per cent casein groups. As compared with the R.Q. values between 0.5 km/hr and 2.5 km/hr in running speeds, R.Q. at 2.5 km/hr was significantly higher than that at 0.5 km/hr in the 20 per cent casein group (P<0.01) and 8 per cent casein group (P<0.05).
Figure 5 shows oxygen requirement for running 100 m at different speeds. The oxygen requirement tended to decrease as the speed increased from 0.5 km/hr to 1.5 or 2.0 km/hr, but at higher speeds from 2.0 km/hr to 2.5 km/hr, oxygen requirement for running 100 m tended to increase, although no significance was found. Also the oxygen requirement tended to be higher in the 20 per cent casein group than in the 8 per cent casein group from 0.5 km/hr to 1.5 km/hr of running speeds.

Table 4 shows the value of \((T - R)/R\) in each speed, in which \(R\) means for resting metabolism and \(T\) means for total energy consumption. This ratio may be regarded as corresponding to R.M.R. for human subjects (16,17). In either diets

### Table 4. \((T^a - R^b)/R\) and grade of exercise for the rats fed diets of various protein levels.

| Protein level | 8%   | 20%   |
|---------------|------|-------|
| No. of sample | 5    | 5     |
| Grade of exercise (km/hr) |      |       |
| 0.5           | 0.76 | 0.99  |
| 0.8           | 1.00 | 1.25  |
| 1.0           | 1.09 | 1.22  |
| 1.2           | 1.09 | 1.36  |
| 1.5           | 1.28 | 1.49  |
| 2.0           | 1.83 | 1.77  |
| 2.5           | 2.31 | 2.38  |

\(^a\) Total energy consumption (kcal) for exercise.

\(^b\) Energy consumption (kcal) at rest. For the \((T - R)/R\), see text.

### Table 5. Oxygen uptake at rest and during exercise (1.0 km/hr).

| Prot. level | Total running (km) | 7 (days) | 14 (days) | 21 (days) | 28 (days) |
|------------|--------------------|----------|-----------|-----------|-----------|
| 8%         |                    | 8.7      | 23.2      | 39.6      | 56.0      |
| O₉ uptake at resting metabolism (ml/G/min) | 0.77 ±0.09 | 0.74 ±0.06 | 0.76 ±0.06 | 0.75 ±0.07 |
| O₂ uptake during exercise (ml/G/min) | 1.57 ±0.13 | 1.54 ±0.09 | 1.48 ±0.13 | 1.49 ±0.13 |
| O₂ requirement during exercise (ml/G/min) | 0.80 ±0.21 | 0.80 ±0.09 | 0.70 ±0.10 | 0.74 ±0.26 |
| 20%        |                    | 0.75 ±0.07 | 0.78 ±0.07 | 0.79 ±0.07 | 0.69 ±0.10 |
| O₂ uptake at resting metabolism (ml/G/min) | 1.82 ±0.25 | 1.81 ±0.55 | 1.78 ±0.24 | 1.51 ±0.26 |
| O₂ uptake during exercise (ml/G/min) | 1.07 ±0.23 | 1.03 ±0.45 | 0.99 ±0.27 | 0.82 ±0.23 |

\(^a\) The difference between these values (one on 7 days and the other on 28 days) is significant at \(P<0.05\).
the ratio was greater at higher speeds and at each speed the value of the ratio
tended to be slightly lower for the 8 per cent casein group, although no significant
difference was found statistically.

To clear up the effect of training on oxygen requirement, the measurement of
oxygen requirement during exercise at 1.0 km/hr for 30 min was carried out every
week on the rats of group II at 1 p.m. after starvation for about 15 hr. The
results obtained are shown in Tables 5 and 6. In the 20 per cent casein group,
oxygen uptake in 4th week was significantly lower than that in the 1st week \( P < 0.05 \). Also oxygen uptake for 100 m running showed from 10.94 ml/G in the
1st week to 9.04 ml/G in the 4th week. The difference between these values was
significant at \( P < 0.05 \). But for the 8 per cent casein groups, the effects of training
using this method were not found.

Table 6. Oxygen uptake and oxygen requirement during
100 m running in each week.

| Prot. level | Total running (km) | 7 (days) | 14 | 21 | 28 |
|-------------|--------------------|---------|----|----|----|
| O₂ uptake during exercise (ml/G/100 m) | 9.41 ± 0.80 | 9.25 ± 0.56 | 8.88 ± 0.78 | 8.93 ± 0.75 |
| O₂ requirement during exercise (ml/G/100 m) | 4.81 ± 1.24 | 4.79 ± 0.54 | 4.34 ± 0.61 | 4.44 ± 0.50 |
| O₂ uptake during exercise (ml/G/100 m) | 10.94 ± 1.49 | 10.86 ± 3.30 | 10.68 ± 1.42 | 9.04 ± 1.54 |
| O₂ requirement during exercise (ml/G/100 m) | 6.40 ± 1.30 | 6.19 ± 2.72 | 5.93 ± 1.63 | 4.91 ± 1.40 |

* The difference between these values (one on 7 days and the other on 28 days) is
significant at \( P < 0.05 \).

The effects of training on carbon dioxide excretion were difficult to evaluate
only a slight decrease was found during 4 weeks training in both 8 and 20 per cent
casein diet groups.

**DISCUSSION**

With a treadmill specially designed for rats, exercise of gradually increasing
intensity was loaded on the rats. In these experiments oxygen uptake and oxygen
requirement during exercise were measured in order to study the influence of the
exercise on energy metabolism and to explore the effect of training on it. SUZUKI
et al. (18) suggested that in the white rat, the effects of exercise either performed in
the day time or night time did not differ. In this experiment the exercises were
performed in day time.

Food intake and body weight gain of the rats fed in either the 8 per cent or
20 per cent casein groups were decreased by exercise. This result coincides with that found previously (7–10, 19, 20).

The results obtained here showed that suspension exercise resulted in increased food intake and body weight. Especially, the growth of the exercise rats in the 20 per cent casein group returned to normal from the 6th day of the rest period. These results seem to support our view that exercise which suppressed food intake and inhibited body weight increase, might generally be a kind of stress to animals. The food intake per body weight was found almost the same in the exercise and control groups.

There was, however, difference in the composition of bodies in these two groups. The ratio of protein to fat in body was more increased in the exercise group (11). The details of these results will be presented in the near future.

There was not much difference in resting metabolism between the 8 per cent and 20 per cent casein groups. The oxygen requirement during exercise in both diet groups increased steadily as the running speed rose. It was also observed there was a tendency for a linear relationship between the oxygen requirement during exercise and running speeds at lower speeds, but at higher speeds, oxygen requirement showed a marked increase surpassing anticipations based on a previous relationship. OGASAWARA reported that the oxygen requirement for running was proportional to the running speeds in the low speed category (21), but YAMAOKA has noted that the rate of increase of the requisite energy is not a linear relation, but an exponential function (22, 23).

On the other hand, the 8 per cent casein group showed smaller oxygen requirement at lower speeds (0.5 km/hr through 1.5 km/hr) than did the 20 per cent casein group, but their oxygen requirement showed a steep rise at higher speed, more than 2.0 km/hr, exceeding that of the 20 per cent casein group.

In these experiments, there were no significant difference between the 8 per cent and 20 per cent casein groups in the respiratory quotient (R.Q.) at each running speed. As compared with the R.Q. values between 0.5 km/hr and 2.5 km/hr in running speeds, R.Q. at 2.5 km/hr was significantly higher than that at 0.5 km/hr in both casein groups.

At higher speeds, the 20 per cent casein group showed a higher R.Q. It seems that the animals in these groups could run well even with low oxygen consumption. The rats in the 8 per cent casein group, however, showed relatively lower R.Q. and more oxygen consumption in spite of the heavy exercise. It is well known that the higher the dependence upon carbohydrate in the energy metabolism of exercise rats, the better the efficiency of the energy production (24). From these facts, it was suggested that the rats in the 20 per cent casein group might have much higher adaptabilities in the metabolic changes to the exercise load, than those of the 8 per cent casein group.

At lower speeds, it is very difficult to explain the results because the difference of R.Q. was not apparent between the 8 per cent and 20 per cent casein groups.
From the results in which the 8 per cent casein group showed smaller oxygen requirement and slightly higher R.Q., it can be taken to indicate the higher burning rates of carbohydrate in the case of the 8 per cent casein group. Of course, the difference of body weight between the 8 per cent and 20 per cent casein groups should be taken into account. Also from these results, it was suggested that the rats of the 8 per cent casein group might produce more calories per unit of oxygen than that the rats of the 20 per cent casein group did, so that less oxygen consumption might be required for sufficient energy to be produced.

**Atzler** and **Herbst** observed 3.08 km/hr as the optimum speed for walking (25). Also **Okuyama** reported that it was 3.6 km/hr for Japanese (26). In another experiment **Yamaoka** (22, 23) has also determined the optimum speeds in swimming, 3.0 km/hr for crawl and 1.98 km/hr breaststroke, in each of which the oxygen requirement for 100 m swim was the smallest. Also for the rats, there might be the optimum speed for running, even if it is forced running on a treadmill. In the present experiments, the oxygen requirement during 100 m running gradually decreased as the speed increased from 0.5 km/hr through 1.5 or 2.0 km/hr, and then increased slightly. The measurement of oxygen requirement during over 2.5 km/hr running was impossible because the animals only ran for a few minutes at such a speed in the apparatus. These results of the oxygen requirement during 100 m running seemed to indicate that the optimum speed for the rat weighing about 300 g might be between 1.5 and 2.0 km/hr.

In the 8 per cent casein group there was no difference between the oxygen uptake during 1.0 km/hr running in the 1st week and that of the 4th week whereas in the 20 per cent casein group there was a significant decrease in oxygen uptake. Since the 8 per cent and 20 per cent casein groups were exactly the same age and had identical training conditions, such a difference may be attributed to the difference of the protein level in their diets or to their body weight. In view of the fact that it was very difficult to find out any difference in oxygen requirement at 1.0 km/hr between the 8 per cent and 20 per cent casein groups, in spite of the difference in body weight, it is assumed that the difference of the effect of training is mainly ascribable to the difference in the protein level in diets.

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ENERGY METABOLISM OF RATS DURING EXERCISE

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