EFFECT OF VOLUNTARY EXERCISE ON PHYSIOLOGICAL FUNCTION AND FEEDING BEHAVIOR OF MICE ON A 20% CASEIN DIET OR A 10% CASEIN DIET

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Summary The effects of voluntary exercise on growth and food intake, body composition, organ weight, and fatty acid composition of adipose tissue of mice fed on a 20% casein diet or a 10% casein diet were examined. The weight gain was greater for the 20% casein nonexercise group (20% NE) than that for the 20% casein exercise group (20% E), 10% casein exercise group (10% E) and 10% casein nonexercise group (10% NE). There were no significant differences between the groups except the 20% NE. In 20% E and 10% E, body fats decreased markedly. On the other hand, a very high ratio of protein was present in the body composition of both groups. In the 20% and 10% casein diet groups, food intake was increased by voluntary exercise, but there was no significant difference between 10% E and 10% NE except occasional periods during these experiments. After 6 weeks of age, 10% E had a tendency to undertake more voluntary exercise than 20% E, though the difference was not statistically significant. Development of the heart and gastrocnemius muscles was accelerated by voluntary exercise and epididymal fat tissue was markedly decreased.

Keywords voluntary exercise, low protein diet, growth, food intake, calorie intake, body composition

In recent years, it has been emphasized that physical exercises are necessary to maintain physical fitness and to prevent obesity or senescence. It seems that the main factors affecting physical fitness and health are dietary regimen and physical exercise. The problem of the relationship between nutrition and health or life-span has been studied by many researchers among whom the studies of MACCAY *et al.*(1) and BERG and SIMMS(2) were excellent. Nevertheless, there are still many uncertainties with regard to the relationship between physical exercise and longevity

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or health from a nutritional point of view. Notably, there are very few reports about the correlation between the protein: calorie ratio in diets, physical exercise and health. It is, however, an interesting research problem.

ANDIK *et al.* reported that rats fed a 4.3% protein diet transferred into the cold not only survived during the experimental period but also gained weight, though rats on a 4.3% protein diet at room temperature failed to gain weight and all died towards, or shortly after the end of the 6th week (3). MEYER and HARGUS noted that rats fed a 10% casein diet gained more weight in the cold (2°C) than at 26°C, whereas the reverse was observed in rats fed a 25% casein diet (4). It may be postulated that a cold environment allows animals to adapt to low protein diets because the increased energy expenditure increases food intake and consequently raises the protein content.

In addition, physical exercises possible increase energy expenditure and food consumption. Therefore, it is expected in this context, that physical exercise, especially voluntary exercise, may be able to modify the nutritional effect of dietary protein levels in an animal. The present paper describes an investigation undertaken as basic research for the effect of voluntary exercises on feeding behavior and physiological function.

EXPERIMENTAL

Animals and diets. Weaning, male JCL:ICR mice (Japan Clea Inc., Tokyo), weighing 13 to 15 g, were used in this experiment. For the experimental diet, the 20% casein diet consisted of 20% casein, 56% potato starch, 13% sucrose, 5% soybean oil, 4% salt mixture, 1.8% vitamin mixture and 0.2% choline chloride. The 10% casein diet was the same composition as the 20% casein diet except for 10% casein, 64% potato starch and 15% sucrose. The two diets were isocaloric.

All mice were housed individually in wire net cages and fed a 20% casein diet for one week. After this period, they were divided into four groups, designated nonexercise group (20% NE) or exercise group (20% E) on the 20% casein diet and nonexercise group (10% NE) or exercise group (10% E) on the 10% casein diet. The number of mice in each group was ten. Both exercise groups were housed individually in wire net cages with a revolving cage. The animals were kept individually on the experimental diets for about 60 days. Throughout the experiment, fresh diet and water were supplied *ad libitum*. Lighting was regulated to provide 12 hr light and 12 hr darkness. Room temperature was maintained at $24^{\circ}\text{C} \pm 1^{\circ}\text{C}$.

The weight of every animal was measured on alternate days in the afternoon. The food intake was measured twice a week.

Measurement of the amount of voluntary exercises. A revolving cage (circumference: 44 cm, width: 6 cm) allowed the animals to exercise voluntarily. The amount of voluntary exercise (running distance) was measured by an electric counter, which records the rotation numbers of the revolving cage.

Removal of the organs and tissues. After the body length and tail length were measured, all mice were killed by ethyl ether anesthesia, and dissected. The heart, liver, kidneys, adrenals, stomach, spleen, epididymal fat tissue and gastrocnemius muscle were removed and weighed. At the same time, the length of the femur was measured with precision calipers. The eviscerated carcass and epididymal fat tissue were frozen at -20°C in a refrigerator until required for the analyses of carcass composition and fatty acid composition, respectively.

Analysis of eviscerated carcass composition. Water content was determined by the freeze-drying method. The crude fat was analyzed by the Soxhlet method. The nitrogen was analyzed according to the semimicro-Kjeldahl method.

RESULTS

The effects of voluntary exercise on the weight gains of mice fed a 20% or 10% casein diet

The weight gains of four groups during 5 weeks are shown in Fig. 1. The 20% NE group had a larger weight gain than the other three groups. The weight gains of the 20% E group and 10% E group were similar and that of the 10% NE group was

Fig. 1. Growth curves. ○, 20% E; ●, 20% NE; □, 10% E; ■, 10% NE. a: $p < 0.05$ Between 20% NE and 10% NE.

the smallest of the four groups. The 20% NE group had significantly larger body weights than the 10% NE group from 6 to 7 weeks of age ($p < 0.05$), but apart from that period no significant differences between the body weights of any group were observed.

Amount of voluntary exercise of mice fed a 20% or 10% casein diet

As shown in Fig. 2, until 6 weeks of age the running distance was short in both groups. Whereas after 6 weeks of age, the running distances showed a tendency to be twice as far as at 6 weeks of age in both groups. The running distances of the 10% E group had a tendency to be longer than the 20% E group. It was found that the running distances for both groups were longer in the dark than in the light.

Fig. 2. The amount of voluntary exercise in light and dark. ○, 20% E (light); ●, 20% E (dark); □, 10% E (light); ■, 10% E (dark)

The effect of voluntary exercise on food intake, calorie intake and protein intake

The food intake, calorie intake and protein intake of mice are shown in Table 1 and Fig. 3. During the experimental period, the average daily food intake of both diet groups was larger in the exercise groups than in the nonexercise groups. Food intake per 100 g body weight was similar. The 20% E group was significantly greater than the 20% NE group ($p < 0.01$), but there were no significant differences between the 10% E and 10% NE groups. When food intake per 100 g body weight of the 20% casein diet groups was compared with the 10% casein diet groups, the 10% E and

Table 1. Effect of voluntary exercise on food intake, calorie intake and protein intake.

Group	Initial body weight (g)	Final body weight (g)	Average daily food intake		Average daily calorie intake		Average daily protein intake	
			g	g/100 g B.W.	Cal	Cal/100 g B.W.	g	g/100 g B.W.
20% E	15.7 ± 1.0 (10)	31.2 ± 3.2 (8)	5.1 ± 0.6	21.5 ± 1.3 ^a b'	18.7 ± 2.1	77.6 ± 4.2 ^a b'	1.02 ± 0.10 ^a b	4.28 ± 0.28 ^a b'
20% NE	15.5 ± 1.3 (10)	35.8 ± 3.1 (8)	4.6 ± 0.5 ^a	19.2 ± 0.9 ^a	17.7 ± 1.1	69.3 ± 3.2 ^a	0.92 ± 0.10 ^a	3.81 ± 0.16 ^a
10% E	15.9 ± 1.8 (10)	31.7 ± 2.5 (8)	5.5 ± 0.5	24.6 ± 2.6	20.1 ± 1.8	89.7 ± 8.8	0.55 ± 0.06	2.45 ± 0.22
10% NE	15.7 ± 0.7 (10)	31.7 ± 2.1 (8)	5.1 ± 0.5	23.0 ± 2.2	18.5 ± 1.9	83.5 ± 8.3	0.51 ± 0.05	2.31 ± 0.21

20% E, 20% casein diet exercise group; 20% NE, 20% casein diet nonexercise group; 10% E, 10% casein diet exercise group; 10% NE, 10% casein diet nonexercise group.

Number of mice is in parenthesis.

^a $p < 0.05$, ^a $p < 0.01$ Between both diets. ^b $p < 0.05$, ^b $p < 0.01$ Between nonexercise and exercise groups.

Fig. 3. Effect of voluntary exercise on food intake. $\circ$, 20% E; $\bullet$, 20% NE; $\square$, 10% E; $\blacksquare$, 10% NE. a: $p < 0.05$, a': $p < 0.01$ Between both diet groups. b: $p < 0.05$, b': $p < 0.01$ Between non-exercise and exercise group.

10% NE group exceeded the 20% E and 20% NE groups (10% E vs. 20% E; $p < 0.01$, 10% NE vs. 20% NE; $p < 0.01$). In the results of food intake twice weekly, as shown in Fig. 3, food intake in both groups tended to be greater in the exercise groups than in the nonexercise groups and significant differences between both diet groups or nonexercise and exercise groups were observed for several periods. Food intake per 100 g body weight was similar. Calorie intake per 100 g body weight of the 20% E group was significantly greater than that of the 20% NE group ($p < 0.01$) and there were significant differences between the 20% casein diet groups and the 10% casein diet groups (20% E vs. 10% E; $p < 0.01$, 20% NE vs. 10% NE; $p < 0.01$). Protein intake was significantly greater in the 20% E group than in the 20% NE group ($p < 0.05$); protein intake per 100 g body weight was similar. When protein intake and protein intake per 100 g body weight of the 20% casein diet groups were compared with those of the 10% casein diet groups, the 20% E and 20% NE groups were found to greatly exceed the 10% E and 10% NE groups.

The effect of voluntary exercise on the carcass composition of mice fed a 20% or 10% casein diet

Table 2 shows the contents of crude proteins, crude fats and water in the four groups. The exercise groups had lower percentages of body fat and higher percentages of protein and water than the nonexercise groups. These differences were statistically significant.

The effect of voluntary exercise on body length, tail length, bone length and organ or tissue weights

As shown in Table 3, there were no significant differences in body, tail and

Table 2. Effect of voluntary exercise on the body composition.

Group	Final body weight (g)	Eviscerated carcass weight (g)	% of wet eviscerated carcass		
			Protein	Fat	Water
20% E	31.2 ± 3.2	22.0 ± 2.2	20.4 ± 0.9 ^b	9.6 ± 3.4 ^b	62.5 ± 2.4 ^b
20% NE	35.8 ± 3.1	25.1 ± 2.5	17.2 ± 1.4	21.2 ± 2.4	53.8 ± 4.9
10% E	31.7 ± 2.5	21.2 ± 2.1	20.4 ± 2.0 ^b	11.2 ± 3.7 ^b	60.9 ± 4.2 ^a
10% NE	31.7 ± 2.1	21.7 ± 1.6	17.8 ± 1.2	20.8 ± 3.6	55.7 ± 3.0

Values are mean ± S.D. of eight mice.

^a $p < 0.05$, ^b $p < 0.01$ Between nonexercise and exercise groups.

Table 3. Effect of voluntary exercise on weights of organs, muscle and fat tissue.

		20% casein diet				10% casein diet			
		Nonexercise		Exercise		Nonexercise		Exercise	
Heart	(g)	0.12 ± 0.01	(0.35 ± 0.03)	0.15 ± 0.04	(0.47 ^{b'} ± 0.09)	0.12 ± 0.01	(0.38 ± 0.04)	0.13 ± 0.02	(0.41 ± 0.05)
Lung	(g)	0.17 ± 0.01	(0.48 ± 0.05)	0.18 ± 0.02	(0.58 ^{b'} ± 0.03)	0.17 ± 0.01	(0.55 ± 0.05)	0.18 ± 0.04	(0.60 ± 0.17)
Kidney	(g)	0.46 ^{a'} ± 0.06	(1.29 ± 0.13)	0.55 ^a ± 0.15	(1.77 ^{a b'} ± 0.35)	0.38 ± 0.04	(1.19 ± 0.13)	0.41 ± 0.06	(1.33 ± 0.16)
Spleen	(g)	0.08 ± 0.21	(0.21 ± 0.04)	0.11 ^{a b} ± 0.04	(0.36 ^{a b'} ± 0.09)	0.07 ± 0.01	(0.23 ± 0.04)	0.08 ± 0.02	(0.25 ± 0.06)
Liver	(g)	1.34 ^{a'} ± 0.18	(3.77 ± 0.48)	1.20 ± 0.16	(3.87 ± 0.53)	1.14 ± 0.06	(3.60 ± 0.19)	1.15 ± 0.14	(3.66 ± 0.43)
Adrenals	(mg)	3.0 ^{a'} ± 0.9	(8.2 ^{a'} ± 2.1)	3.3 ± 1.1	(11.1 ± 3.4)	4.2 ± 0.5	(13.3 ± 1.8)	3.8 ± 0.6	(12.4 ± 1.4)
Gastrocne- mius	(g)	0.14 ± 0.04	(0.39 ± 0.09)	0.18 ^b ± 0.02	(0.59 ^{b'} ± 0.07)	0.12 ± 0.02	(0.40 ± 0.07)	0.19 ^{b'} ± 0.03	(0.60 ^{b'} ± 0.09)
Epididymal	(g)	1.23 ^b ± 0.33	(3.41 ± 0.66)	0.58 ^{b'} ± 0.14	(1.85 ^{b'} ± 0.41)	0.89 ± 0.23	(2.79 ± 0.56)	0.53 ^b ± 0.25	(1.57 ^{b'} ± 0.70)
Body length	(cm)	10.2 ± 0.3		10.1 ± 0.3		10.1 ± 0.1		10.0 ± 0.3	
Tail length	(cm)	9.8 ± 0.5		9.5 ± 0.4		9.9 ± 0.4		9.5 ± 0.4	
Femur	(cm)	1.56 ± 0.04		1.60 ± 0.07		1.54 ± 0.05		1.57 ± 0.06	

^a $p < 0.05$, ^{a'} $p < 0.01$ Between both diets. ^b $p < 0.05$, ^{b'} $p < 0.01$ Between nonexercise and exercise. () ; measurement per 100 g body weight. Values are mean ± S.D. of eight animals.

femur lengths among four groups. The 20% E and 10% E groups tended to have larger hearts than the 20% NE and 10% NE groups. Heart weight per 100 g body weight was significantly heavier in the 20% E group than in the 20% NE group (20% E vs. 20% NE; $p < 0.01$). With regard to the kidneys, those of the 20% casein diet

groups were significantly larger than those of the 10% casein diet groups (20% E vs. 10% E; $p < 0.05$, 20% NE vs. 10% NE; $p < 0.01$). The spleens of the 20% E group were significantly greater than those of the 20% NE group ($p < 0.05$); when the 20% E group was compared with the 10% E group, there was a significant difference ($p < 0.05$). The adrenals of the 10% casein diet groups were heavier than those of the 20% casein diet groups. For adrenals and adrenals per 100 g body weight, there were significant differences between the 20% NE and 10% NE groups ($p < 0.01$). When epididymal fat tissues of the 10% E and 20% E groups were compared with those of the 10% NE and 20% NE groups, epididymal fat tissues of the E groups were significantly less than those of the NE groups (20% E vs. 20% NE; $p < 0.01$, 10% E vs. 10% NE; $p < 0.01$). The proportion of decrease in fat tissue in the 20% E group was 53% and that in the 10% E group was 40%. Fat tissues per 100 g body weight showed similar results. In both diet groups, the gastrocnemius muscle showed markedly heavier values in the exercise groups than in the nonexercise groups.

DISCUSSION

Previously, it has been found that the body weight gains of rats are decreased by training (5, 6). In the present study, the 20% E group had smaller body weight gains than the nonexercise group. As for the reason for reduced body weight gains, it is thought that the deposition of fat was less because the calorie consumption was increased by exercise. The strongest proof appears to be that, in the present study, the carcass fat content and adipose tissue were at low levels in the exercise group, whereas there was no difference between the body weight gains of the 10% E and NE groups. This may be due to the fact that the deposition of fat in adipose tissue was less in the 10% NE group than in the 20% NE group and body protein in the 10% E group was significantly greater than in the 10% NE group. Formerly, there have been observations demonstrating that enforced exercise by treadmill depresses food intake in spite of the increased energy expenditure (7, 8). Nevertheless, SUZUKI *et al.* (9) have reported that voluntary exercise of rats increased food intake in accordance with the amount of exercise. The present study also showed that, generally, the food intake of the exercise groups tended to be larger than that of the nonexercise groups and, furthermore, that it was larger in the 10% casein diet groups than in the 20% casein diet groups. Moreover, the body weight gains of the 10% E group increased at levels approaching those of the 20% E group. Therefore, it is surmised that a low protein diet at the 10% casein level does not exert a serious influence on the growth of animals if food intake or calorie intake increases. In our experiments, it was observed that body weight gains in all four groups increased rapidly until 6 weeks of age and thereafter became slower. On the other hand, the results showed that the amount of voluntary exercise in both groups increased rapidly after 6 weeks of age. This fact suggests that animals during the development of organs, tissues and bones, namely animals in the growing period, have no

physiological functions adequate for strenuous physical exercise. As mentioned in Results, the amount of physical exercise of the 10% E group tended to be larger than that of the 20% E group after 6 weeks of age. FUGE *et al.* (10) reported that rats fed an 8% casein diet were able to run significantly further than rats fed a 22% casein diet in an all-out exercise test and they suggested the possibility that the lower body weight of rats fed an 8% casein diet may have played a role in enabling them to outrun rats fed a 22% casein diet; more rapid diffusion of lactate from the muscles of the protein-deficient animals may be a factor contributing to their larger capacity for prolonged running. Therefore, factors as mentioned above seem to be the cause of the present experimental result, but a precise explanation could not be obtained.

In the present investigation, it was found that the development of organs and muscles of exercise groups was better than that of nonexercise groups. Particularly, development of the gastrocnemius was accelerated by physical exercise. It has also been reported that the development of organs and bones of rats is retarded by exercise (11). The discrepancy with the above results may be due to disparity of species and the distinction between enforced exercise and voluntary exercise.

As described in Results, it was found that the weight of epididymal fat tissue and the ratio of body fat were decreased markedly by physical exercise. However, SUZUKI and KOYANAGI (11) noted that epididymal fat tissue was not affected by training, although subcutaneous fat tissue, perirenal fat tissue, *etc.* were decreased. In the present investigation, since adipose tissues except for epididymal fat tissue were not examined, the effect of physical exercise on those fat tissues was not shown. The two results on epididymal fat tissue above contradict each other; this discrepancy may result from the amount of exercise, disparity of species, or the distinction between voluntary and enforced exercise, *etc.*

The facts described above suggested that animals fed a low protein diet at the 10% casein level may easily adapt to physical exercise and increasing food intake. At the same time, it is expected that physical exercise may become a factor contributing to the growth and development of animals in a state of malnutrition. Currently, the problems concerned with more severe protein-deficient diets are being examined.

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