Effect of Voluntary Exercise on 3′-Methyl-4-Dimethylaminoazobenzene-Induced Hepatomas in Male Jc1:Wistar Rats (43655)

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Abstract. **The effect of voluntary exercise on 3′-methyl-4-dimethylaminoazobenzene (3′-Me-DAB)-induced hepatomas was investigated in male Jc1:Wistar rats. Beginning at 10 weeks of age, animals were divided into two groups (sedentary and exercise) and housed in individual cages. Food intake and wheel exercise were automatically controlled in the cages of the exercise group. Body weights were monitored throughout the study. Food availability was controlled in order to equate length and weight gain. From 27 weeks to termination of the study at 62 weeks, all animals were administered 3′-Me-DAB in the diet at a dose level of 0.0177 g/day/kg body wt. All animals were sacrificed at 62 weeks of age. The incidence of hepatomas was significantly lower in the exercise group as compared with the sedentary group (0% and 65%, respectively). Liver weight was significantly greater in the exercise group compared with sedentary animals without hepatomas. The weight of epididymal fat pads was significantly lower in the exercise group. Serum alkaline phosphatase was significantly higher in the exercise group as compared with the sedentary group. Serum γ-glutamyl-transpeptidase levels were higher in the sedentary group than in the exercise group. In addition, γ-glutamyl-transpeptidase levels were significantly higher in sedentary animals with hepatomas than in sedentary animals without hepatomas. These results demonstrate that voluntary exercise decreases 3′-Me-DAB-induced hepatomas and that this decrease is associated with an increase in serum alkaline phosphatase and a decrease in serum γ-glutamyl-transpeptidase levels.** [P.S.E.B.M. 1993, Vol 204]

A pioneering study by Sivertsen and Dahlstrom (1), one of the many subsequent studies since 1921, indicated an inverse relationship between physical activity and cancer mortality in Minnesota residents. The relationship between physical activity and tumor incidence has also been the subject of several reviews (2–4). All these studies concluded that exercise may be effective in suppressing the development of cancer, especially colon cancer in males (5–7) and reproductive organ cancers in females (8). There are, however, several methodological problems in epidemiological research. For example, the total amount of exercise in an entire lifetime cannot be measured in epidemiological research. In addition, the effect of exercise on tumor development cannot be determined because of the existence of many other confounding factors related to tumor development.

In laboratory animal studies, results varied with the type of exercise. For instance, in the case of carcinogen-induced mammary tumors in rats, tumorigenesis decreased with voluntary wheel exercise (9), but increased with enforced treadmill exercise (10). Although voluntary exercise is generally desirable in animal experiments, it has shortcomings in that it produces large individual differences in the amount of exercise as well as a rapid decrease in the amount of exercise as the rats become older (11–13). An additional point that must be considered is the amount of food intake. Because there is a close relationship between the amount of available food, body weight (obesity), and tumor development (14–19), it is necessary to control food intake

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and body weight gain in order to interpret the results properly. Also, since food can become available as a result of food-seeking activity in natural ecosystems, it is also desirable that animals be fed after exercise. In order to minimize the effects of these variables in animal experiments, we have developed an animal maintenance system that controls the amount of exercise, amount of food intake, and feeding times (20).

We reported recently that voluntary wheel exercise suppresses spontaneous hepatomas (12) and benzidine-induced hepatomas (21, 22) in Jc1:ICR mice. In another study on the relationship between voluntary exercise and azoxymethane-induced colon carcinogenesis in rats, no significant difference was found in the incidence of liver neoplastic nodules, but a significant inhibition of liver cell foci by exercise was established (23).

In the current study, we investigated the effect of voluntary exercise on 3′-methyl-4-dimethylaminoazobenzene (3′-Me-DAB)-induced hepatomas in rats raised under controlled conditions for the amount of voluntary exercise, food availability, and 3′-Me-DAB administration, using an animal maintenance system developed in our laboratory (20). Blood biochemistry was also examined in order to elucidate the characteristics of hepatomas.

Materials and Methods

Animals used in the experiment were 10-week-old male Jc1:Wistar rats supplied by Clea Japan, Inc. (Tokyo, Japan). They were randomly divided into two groups: the exercise group (n = 19) and the sedentary group (n = 17), each of which was raised in a single cage. At the beginning of the experiment, there was no difference in the body weights of the animals in the two groups (exercise group, 280 ± 3.2 g; sedentary group, 281 ± 4.3 g). In the raising and holding rooms, the temperature was maintained at 22°C and humidity at 55%. The room was illuminated during the day time (0600–1800 hr), and the light was turned off at night (1800–0600 hr). Laboratory chow (OA-2) supplied by Clea Japan Inc. was used as raising food. 3′-Me-DAB (Tokyo Kasei Kogyo Co., Ltd., Tokyo) was mixed with OA-2 diet at 0.06% and administered orally (24).

As indicated above, animals in the exercise group were engaged in exercise under the animal maintenance system developed by Ikuyama *et al.* (20). Voluntary wheel exercise was performed in a cage measuring 24 cm × 34 cm (Figs. 1 and 2). In this protocol, when the rats achieved the preset rotation frequency, a premeasured amount of food from the storage area was automatically released into the feeding box. Animals were allowed to exercise freely between the ages of 10 and 17 weeks in order to accustom them to wheel exercise. In our previous experiment, rats of 52 weeks of age were easily able to complete the wheel exercise of 5000

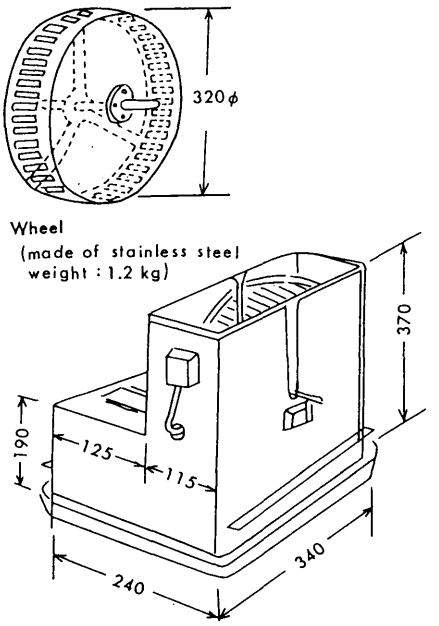

Figure 1. Sizes of the house cage and the wheel.

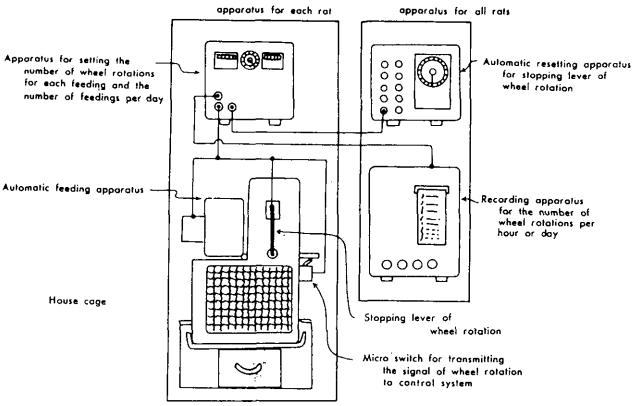

Figure 2. The outline of relation of each part in the apparatus.

rotations/day (20). On the bases of this experiment, during the period from 17 to 24 weeks of age, the preset rotation frequency was increased gradually to 3000 rotations/day, at which point it was thenceforth maintained until the end of the experiment at 62 weeks. In the sedentary group, rats were raised in cages identical in size to that of the exercise group. Since Clean-chip (a nonprickly, high-quality brand of sawdust, Clea Japan Inc.) was used as a floor covering for the cage in both exercise and sedentary groups, the level of physical activity inside the cage by the animals was considered to be greater than that in cages in which wire mesh is used to cover the cage floor.

In order to equalize body size and weight and to avoid retardation of bone development, all animals in both groups were given food (OA-2) *ad libitum* during the period from 10 to 16 weeks of age. In our previous experiment, when food intake was 17 g/day (119

g/week), the rats in the exercise group were able to complete the wheel exercise of 5000 rotations/day (20). Therefore, food availability was limited from the 17th week of age onward to 119 g/week for the exercise group and 98 g/week for the sedentary group; the amount for the latter group was further reduced to 91 g/week in the 24th week. As a result, the average body weights of animals in the two groups were almost the same at 26 weeks of age (398 ± 14.3 g in the exercise group and 398 ± 18.9 g in the sedentary group). From that point until the end of the experiment at 62 weeks of age, food availability was maintained at 119 g/week for rats in the exercise group and 91 g/week for those in the sedentary group. Rats in the sedentary group were fed automatically at 1800 hr every day by means the system developed in our laboratory.

During the period between 27 weeks of age and the end of the experiment (62 weeks of age), 3′-Me-DAB was administered in the chow to the animals. In order to equalize the 3′-Me-DAB dose per body weight for animals in the two groups, the following protocol was adopted. Rats in both the exercise and sedentary groups were given 3′-Me-DAB at a dose rate of 0.1239 g/kg body wt/week (e.g., in the case of animals weighing 440 g, 91 g/week of food containing 0.06% 3′-Me-DAB were administered). The amount of 3′-Me-DAB to be added to food was calculated on the basis of body weight as measured once a week. Then the calculated amount of 3′-Me-DAB was added to the diet to bring the total food allowance to 119 g/week and 91 g/week for the rats in the exercise and sedentary groups, respectively. These weekly allowances were divided by seven to determine the amount of food to be administered daily.

The rats were sacrificed at 62 weeks of age after 24 hr of fasting. Macroscopic examination was used to check for the presence of abnormal tissues. Then histopathological examination of hematoxylin and eosin-stained samples was conducted under a microscope for identification of hepatomas. Macroscopic and histopathological examinations were made under the condition where it was unable to distinguish between rats of the exercise group and sedentary group.

A previous study of 3′-Me-DAB-induced hepatomas in rats revealed large amounts of γ-glutamyl transpeptidase (γ-GTP) in undifferentiated tumor tissues and alkaline phosphatase (ALP) in differentiated tumor tissues (25). Therefore, in order to evaluate the characteristics of hepatomas, γ-GTP and ALP in serum were measured by a modified Orlowski colorimetric method (26) and Bessey-Lowry colorimetric method (27), respectively.

Student's *t* test was used to determine statistical significance between sedentary and exercise groups.

Results

Figure 3 shows the body weights of sedentary and exercise groups from Week 10 through termination of the study. Table I summarizes physical characteristics including body weight, body length, and liver and epididymal fat pad weights. The body weights of animals in the exercise and sedentary groups were similar from the 27th to 56th weeks of age. However, from the 57th to 61th weeks of age, rats in the sedentary group weighed significantly more than those in the exercise group (Fig. 1). The reason for this remains unclear. No difference was found in body length (from tip of nose to anus) between the two groups at the time of sacrifice. There was also no difference between the liver weights in the two groups. However, animals in the sedentary group that developed hepatomas had liver weights differing greatly from the average. This may be due to the fact that three of the rats in the sedentary group developed large hepatomas measuring 10–42 mm in diameter. Animals in the sedentary group without hepatomas had significantly smaller liver weights than did those in the exercise group. The weight of the epididymal fat pads was significantly greater in the sedentary group than in the exercise group, independent of the development of hepatomas.

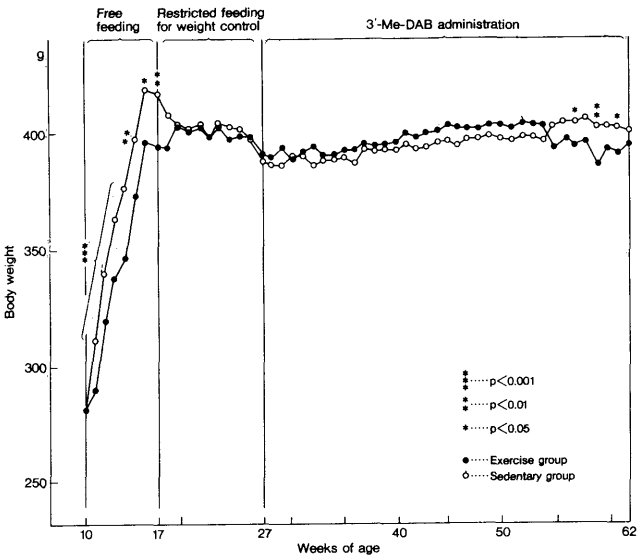

Figure 3. Effect of restricted feeding and exercise on body weight of animals administered 3′-Me-DAB.

Table II summarizes the results of hepatomas. None of the animals in the exercise group developed 3′-Me-DAB-induced hepatomas, whereas 64.7% of animals in the sedentary group developed hepatomas.

The level of serum ALP in the exercise group was significantly higher than that in the sedentary group (Table III). The serum γ-GTP level of sedentary rats with hepatomas was significantly higher than that of either sedentary rats without hepatomas or rats in the exercise group (Table III).

Discussion

Previous studies in animal models on the relationship between exercise and cancer development indicate

Table I. Physical Characteristics of the Exercise and Sedentary Groups of Male Jc1:Wistar Rats[a]

Experimental group	Animals (*n*)	Body wt (g)	Body length (cm)	Liver wt (g)	Epididymal fat pad wt (g)
Exercise	19	386 ± 16.6	23.9 ± 0.36	16.0 ± 1.51	3.3 ± 0.61
Sedentary[b]	16[c]	392 ± 13.7	23.9 ± 0.34	15.8 ± 5.65	5.4 ± 0.92[d]
With hepatoma	10	389 ± 13.4	23.8 ± 0.35	17.4 ± 6.69	5.7 ± 0.99[d]
Without hepatoma	6	397 ± 13.7	24.0 ± 0.32	13.0 ± 0.70[d]	5.0 ± 0.70[d]

[a] Values represent means ± SD.
[b] Sedentary group includes animals with and without hepatomas.
[c] One of the sedentary rats died before being sacrificed.
[d] Significantly different from exercise group at $P < 0.001$.

Table II. Incidence of 3′-Me-DAB-Induced Hepatomas in Male Jc1:Wistar Rats

Experimental group	Animals (*n*)	Animals with hepatoma (*n*)	Incidence of hepatoma (%)
Exercise	19	0	0[a]
Sedentary	17	11	64.7

[a] Significantly different from sedentary group at $P < 0.001$.

Table III. Serum Alkaline Phosphatase and γ-Glutamyltranspeptidase Levels in Exercise and Sedentary Groups[a]

Experimental group	Animals (*n*)	ALP (units/liter)	γ-GTP (units/liter)
Exercise	19	303 ± 105.2	2.0 ± 1.27
Sedentary	16	164 ± 48.0[b]	5.8 ± 6.27[c]
With hepatoma	10	169 ± 55.2[b]	8.0 ± 7.08[c]
Without hepatoma	6	155 ± 35.8[b]	2.0 ± 0.63[d]

[a] Values represent means ± SD.
[b] Significantly different from exercise group at $P < 0.001$.
[c] Significantly different from exercise group at $P < 0.05$.
[d] Significant difference between subgroups with and without hepatomas in the sedentary group at $P < 0.05$.

that forced treadmill exercise increased dimethylbenzanthracene-induced mammary carcinogenesis (9), whereas voluntary exercise decreased the nitrosomethylurea-induced mammary carcinogenesis in rats (10). This discrepancy might be attributable to the difference in the nature of carcinogen administered between two experiments. However, it also suggests that attention should be paid to the manner in which animals are exercised, since stress (28) and hormone (29) levels affect the development of cancer.

In the current study, we demonstrated that the development of 3′-Me-DAB-induced hepatomas in rats was completely suppressed by voluntary exercise. The experimental protocols used in this study largely satisfied the necessary conditions for investigating the relationship between exercise and tumor development. In the present study, animals performed exercise voluntarily at night, and ate food after exercise. In natural ecosystems, animals engage in food-seeking activity as long as they live, and so perform exercise regularly throughout their lives. Therefore, the experimental protocols of this study, where the animals were fed after a certain amount of wheel exercise, could be considered very close to that of a natural ecosystem. The results of research conducted under these conditions should, thus, be of considerable importance.

The mechanism of exercise-induced inhibition of hepatocarcinogenesis is remotely understood. Since the body weight gain and the caloric restriction has been reported to be closely related to tumorigenesis (14–19), we planned our experiment so as to equalize the body size of animals in the exercise group and sedentary group; actually, the amount of food administered to the exercise group was about 30% larger than that in the sedentary group. The results obtained under these conditions were contrary to previous reports that the incidence of hepatomas in mice becomes lower when the amount of food is decreased (18). The reason for this is that animals generally increase their level of activity when they become hungry. Limitation of food availability hence increases their level of physical activity within the cage, which might have contributed to the lower incidence of hepatomas in mice in past studies (18). It is, therefore, necessary to consider both energy expenditure and energy intake when investigating factors affecting the development of tumors.

No difference was found in liver weight between the exercise group and sedentary group. However, liver weight in the exercise group was significantly larger than that in sedentary rats without hepatomas, which suggests that increased levels of exercise and food intake increase the load on the liver, thereby accelerating liver functions. The sedentary group had higher epididymal fat pad weights compared with the exercise group. Since 3′-Me-DAB is fat soluble, it is probable to believe that the fatter the rat is, the less effect to the liver there is. Concludingly, it is unconceivable that the greater epididymal fat pad weights induce the development of hepatoma.

In the current study, serum ALP levels in the exercise group were found to be higher than those usually found in Jc1:Wistar rats and significantly higher than those found in the sedentary group. It is possible that the increase of serum ALP level in the exercise group reflects the increased level of exercise and food intake. The average values of γ-GTP level were normal (2.0 units/liter) for animals in the exercise group and in the sedentary group without hepatomas. However, in the sedentary rats that developed hepatomas, γ-GTP levels averaged about 8.0 units/liter and the values for seven rats exceeded 4.0 units/liter. In addition, the rats with the largest hepatomas exhibited the highest levels. This suggests that the excess of serum γ-GTP in the rats with hepatomas is made from the hepatoma cells, and they have undifferentiated characteristics.

In summary, our results suggest that voluntary exercise inhibits liver carcinogenesis and that this inhibition is associated with an increase in serum ALP and decrease in γ-GTP in the animal model.

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