Effects of exercise and of food restriction on adipose tissue cellularity

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Abstract The body weight and fat content of young, growing rats were kept low by regularly performed endurance exercise by the rats or by restriction of their food intake over a period of 14 wk. The cellular character of epididymal fat pads was studied to determine if the reduction in fat was due to a decrease in the number of adipose cells, their size, or both. Compared with the sedentary freely eating control animals, both the exercisers and the sedentary paired-weight animals, which had their food intake restricted in order to maintain their body weights approximately the same as those of the exercisers, had significantly lighter epididymal fat pads ($P < 0.001$). This fat depot in the exercisers contained fewer ($4.46 \pm 0.48 \times 10^6$ vs. $6.89 \pm 0.55 \times 10^6$ cells/pad; $P < 0.001$) and smaller (0.286 ± 0.041 vs. 0.462 ± 0.040 μg of lipid/cell; $P < 0.001$) cells than that in the sedentary freely eating animals. Food restriction also resulted in a significant reduction in adipose tissue cellularity ($P < 0.05$). Epididymal fat pads from the calorie-restricted rats had an average of $5.72 \pm 0.33 \times 10^6$ cells and they contained 0.319 ± 0.024 μg of lipid/cell. These results demonstrate that exercise in addition to food restriction in early life is effective in reducing the rate of accumulation of cells in epididymal fat pads of rats.

Supplementary key words appetite · swimming · weight gain · body composition · adipose cells

REGULARLY performed, vigorous exercise has a profound effect on body growth and composition in young animals. Numerous studies have shown that male rats subjected to a program of regularly performed endurance exercise gain weight more slowly and have significantly lower final body weights than freely eating sedentary controls (1–5). The difference between the final body weights of the exercising and the sedentary animals (as much as 120 g [2]) could be explained, to a large extent, by the lower fat content of the carcasses of the exercising animals.

In early life, fat accumulates in epididymal fat pads of rats as a result of an increase in cell number and cell size (6, 7). At approximately 15 wk of age, cell number becomes fixed in this depot, and only cell size changes with further increases in adiposity (6, 7). The finding that exercise caused a reduction in the total-body content of fat in young, growing rats (1, 2) indicates that exercise retards the rate at which adipose tissue cells accumulate or enlarge, or both. The present study was designed, therefore, to test the possibility that exercise, which keeps the total-body content of fat low in young, growing rats, can also reduce the rate at which cells accumulate in epididymal fat pads of these animals. The possibility that exercise can depress cell division in adipose tissue may have interesting implications with respect to the development of severe obesity in later life, since recent evidence shows that, in adult humans, increased cell number plays a more important role in the development of the grossly overweight condition than does cell size (8).

METHODS

Animals, exercise program, and diet

Male Wistar rats (specific pathogen-free CFN rats) were used. At birth, the number of animals per litter was reduced to give each mother a total of five male siblings. At 7 days of age, the rats were obtained from Carworth Farms, and each group of five males, together with their mother, was placed in an individual cage. The rats were immediately divided into three groups closely matched for weight. The formation of these groups was accomplished without separating any of the animals from their mothers.

One group of rats consisted of nine animals, 8 days of age, weighing 26 ± 0.9 g, that were subjected to a

program of swimming over a period of 14–16 wk. They swam, in groups of four or five, in plastic barrels with an internal diameter of 30 cm and a depth of 55 cm. Water temperature was maintained between 34 and 35°C. The animals swam 6 days/wk; the duration of the exercise sessions was progressively increased from 15 to 360 min over a period of 4 wk. The exercisers were maintained at 360 min of daily swimming, 6 days/wk, until they were killed. The exercising group was provided with food and water ad lib.

A sedentary paired-weight group of nine animals that had an average initial body weight of 25 ± 0.6 g had their food intakes restricted so as to result in a rate of weight gain comparable to that of the exercisers. Food restriction before weaning was accomplished by separating the sedentary paired-weight animals from their mothers each day for different periods of time. The third group (average initial body weight of 26 ± 0.5 g) contained nine sedentary freely eating animals that were provided with food and water ad lib.

When the animals reached 15 wk of age, two sedentary animals, one from the paired-weight group and one from the freely eating group, were killed on the same day as the exercising animal paired with them. This procedure was continued for 14 days until all the animals were killed.

After weaning (21 days), the animals' diet contained 18% casein, 49.5% sucrose, 5.75% corn oil, 17.25% lard, 2% brewer's yeast, 2% liver powder, 4% Hegsted salt mixture, and 1.5% vitamin fortification mixture.

The food intake of each animal was measured daily, but only after weaning.

Tissue preparation and assay methods

Rats were killed with ether after a terminal fast of 24 hr, and in the case of the exercisers, 54–78 hr after their last exercise session. Hair was removed as described previously (2). Epididymal fat pads were quickly dissected out, rinsed free of adherent oil droplets in warm isotonic saline, blotted, and weighed. The two fat pads from each animal were then pooled, chopped into a coarse mince with scissors, and mixed thoroughly. Two portions of this mince were taken; one portion was processed for the purpose of making cell counts and the second was used to determine lipid content of adipose mass. The remaining adipose tissue was pooled with the carcass for subsequent analysis of body composition. Preparation of the tissue for cell counts was performed as described by Hirsch and Gallian (9). Cells from approximately 50 mg of adipose tissue were fixed in 30 ml of 2% osmium tetroxide in 0.05 M collidine–HCl buffer, pH 7.4, at 37°C, for a period ranging from 24 to 48 hr. After separation of tissue into free cells and after repeated washings with isotonic saline, the fixed cells were suspended in a known amount of saline and counted with a Celloscope counter (Particle Data, Inc., Elmhurst, Ill.). The suspension of fat cells in filtered saline solution was drawn through a 300-μm orifice in a glass tube. Cells were counted in aliquots of 5 ml. The lipid content of adipose tissue was determined gravimetrically after extraction with alcohol–ether by the method of Entenman (10).

Adipose cell size (lipid content/cell) was calculated using an equation as described previously (6).

In the course of preliminary experiments, difficulty was encountered in our attempts to obtain an even cell suspension while making cell counts. As a result, fat cells were counted in a 150-ml glass beaker placed over a Micro V magnetic stirrer (Cole-Palmer, Chicago, Ill.). A magnetic stirring bar spinning at low speed proved useful in keeping the cells evenly distributed in solution while counting.

Analyses were performed on the carcasses, from which feces and hair had been removed, by a modification (2) of the method of Mickelsen and Anderson (11).

Terminology. The terms fat-free weight and lean body mass are used synonymously in the following sections. They are defined as the weight of the whole carcass minus feces, hair, and total body fat.

Statistical methods. The significance of differences among the three groups was determined by the *t* test for paired observation (12).

Materials

Purified *sym*-collidine buffer was obtained in kit form from Polysciences, Inc., Warrington, Pa. Osmium tetroxide was purchased from Engelhard Industries, Newark, N.J.

Casein, Hegsted salt mixture, brewer's yeast, liver powder, and GBI vitamin fortification mixture were obtained from General Biochemicals, Chagrin Falls, Ohio.

RESULTS

Effects of exercise on weight gain, caloric intake, and body composition

The exercising rats gained weight more slowly and had significantly lower final body weights than the sedentary freely eating animals (Table 1, Fig. 1). The slower rate of weight gain was due solely to an increase in expenditure of calories, as food intake remained unaffected by the exercise (Table 2).

Exercise resulted in a significant decrease in the total-body content of fat (Table 1). At the end of the study, the body fat content of the exercisers was approximately one-fourth of that of the sedentary freely eating controls (Table 1). It can be seen that approximately one-half of

TABLE 1. Weight and composition of carcasses of sedentary and exercising rats

	Sedentary Freely Eating Rats (A)	Exercising Rats (B)	Difference[a]	P	Sedentary Paired-weight Rats (C)	Difference[b]	P
	g	g	g		g	g	
Carcass weight[c]	418 ± 14	260 ± 9	158	< 0.001	266 ± 5	6	NS[d]
Fat weight	102 ± 8	26 ± 2	76	< 0.001	45 ± 2	19	< 0.001
Fat-free weight[e]	313 ± 11	233 ± 9	80	< 0.001	221 ± 4	12	NS

Values are means ± SEM for nine rats.
[a] (A) minus (B).
[b] (C) minus (B).
[c] After removal of hair and feces.
[d] NS, not significant (P > 0.05).
[e] In calculating fat-free weight, carcass protein, water, and minerals were summed.
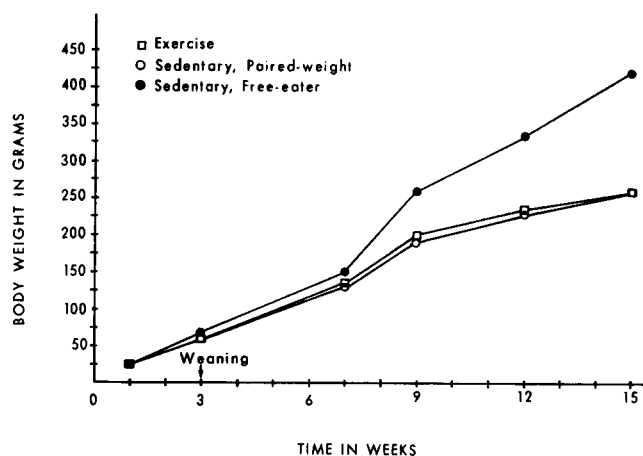

FIG. 1.   Effects of exercise and of food restriction on body weight of rats. Each point is the mean for nine animals.

the difference between the body weights of the swimmers and freely eating sedentary rats can be accounted for on the basis of the lower fat content of the exercisers. In addition to the reduction in body fat, the exercisers had a reduced lean body mass (Table 1).

Effects of food restriction on weight gain and body composition

The sedentary paired-weight animals had their food intakes restricted so as to maintain their body weights approximately the same as those of exercisers; as a result, their body weights compared with those of the free-eaters were significantly reduced (P < 0.001)

TABLE 2. Average daily caloric intake for sedentary and exercising rats after weaning

Sedentary Freely Eating Rats (A)	Exercising Rats (B)	Difference	Sedentary Paired-weight Rats (C)	Difference
		calories		
60 ± 1	59 ± 2	1	34 ± 1	25[a]

Values are means ± SEM for nine rats.
[a] Difference between (B) and (C). P < 0.001.

(Table 1 and Fig. 1). The lower weights of the paired-weight animals, relative to the freely eating sedentary rats, were due to both a significantly lower total fat content (P < 0.001) and a smaller lean body mass (P < 0.001) (Table 1).

Although final weights were essentially the same for the exercisers and sedentary paired-weight controls, their carcasses were markedly different in composition. The carcasses of the sedentary food-restricted controls contained roughly twice as much fat as those of the exercisers (Table 1), and the fat-free weight was slightly less.

Effects of exercise on adipose cell number and size

The exercise program resulted in a significant reduction (P < 0.001) in the size and lipid content of epididymal fat pads, as can be seen in a comparison of the swimmers and the sedentary freely eating controls (Table 3). This fat depot was smaller in exercisers due to decreases in both total number of cells (P < 0.001) and size of cells (P < 0.001). As a result of the marked decrease in cell size, the exercisers had a significantly higher number of cells in their epididymal fat pads than did the sedentary freely eating animals when the results are expressed per gram adipose tissue (P < 0.001).

Effects of food restriction on adipose cell number and size

As shown in Table 3, the food-restricted animals also had significantly lighter epididymal fat pads which contained significantly less fat compared with those of the sedentary free-eaters (P < 0.001). As in the case of the exercisers, a reduction in cell number (P < 0.05) and cell size contributed to make fat pads smaller in the carcasses of the sedentary paired-weight animals.

Comparison of exercised and food-restricted animals

At the end of the study, body weight of the exercisers was approximately the same as that of the food-restricted group (Table 1 and Fig. 1). In spite of this, the size and lipid content of epididymal fat pads in these two groups

TABLE 3. Number and size of adipose cells and lipid content of epididymal pads of exercising and sedentary rats

Group	Epididymal Fat Pad[a]	Adipose Cell Number		Adipose Cell Size	Lipid Content/Pad	
	g	*per g adipose tissue × 10⁶*	*per fat pad × 10⁶*	*µg of lipid/cell*	g	%
Exercising	1.551	3.09	4.46	0.286	1.266	82
	±0.119	±0.41	±0.48	±0.041	±0.140	
Sedentary PW[b]	2.278	2.61	5.72	0.319	1.884	83
	±0.153	±0.25	±0.33	±0.024	±0.130	
Sedentary FE[c]	3.925	1.79	6.89	0.462	3.153	80
	±0.253	±0.16	±0.55	±0.040	±0.196	

Values are means ± SEM for nine rats.
[a] Average value for left and right epididymal fat pads.
[b] PW, paired-weight.
[c] FE, freely eating.

were different. This fat depot in the exercisers was lighter ($P < 0.05$) and contained significantly less fat ($P < 0.01$) (Table 3). To account for the reduction in size, the epididymal fat pads of the exercisers had fewer ($P < 0.01$) fat cells, which were slightly reduced in size compared with those of the sedentary food-restricted rats (Table 3).

DISCUSSION

Fat is stored in adipose tissue cells, which form depots located at various sites in the body. The amount of fat stored in each depot is dependent upon the size and number of its constituent adipocytes. In the present study, exercise, by its ability to keep the total-body content of fat low, significantly reduced the size of epididymal fat pads in young, growing rats. Cells from this fat depot in the exercising animals were found to be fewer in number and smaller in size than those of comparable freely eating controls. It is reasonable to assume that the reduction in adipose tissue cellularity is a permanent effect of exercise because Hirsch and Han (6) and Johnson et al. (7) have clearly shown that epididymal fat pads in rats grow as a result of cell hypertrophy and hyperplasia until the animals are approximately 15 wk of age, when number becomes fixed and only cell size changes with further increases in depot mass. In the present study, the animals were at least 15 wk of age at the time they were killed.

Knittle and Hirsch, by manipulating litter size, were able to vary caloric intake of rats during the suckling period (13). They found that caloric deprivation, in early life, significantly reduced the rate at which fat cells were formed, so that animals raised in large litters and killed at 15 wk of age had 28% fewer cells in their epididymal fat pads than those raised in smaller litters (13). In contrast, the calorie-restricted animals in the present study had a somewhat smaller decrease (17%) in cell number in spite of the fact that these animals experienced

a larger caloric deficit. The difference in body weight between the food-restricted and freely eating control animals in this study was 158 g compared with 96 g for those in the study by Knittle and Hirsch (13). The difference in the degree of depression in adipose tissue cellularity could be related to the time in life when caloric restriction was imposed, suggesting that the events which take place between birth and weaning play an important role with respect to cellularity. Food restriction in our rats was imposed between the 2nd and 3rd wk of life, whereas Knittle and Hirsch (13) studied animals that were subjected to food deprivation from birth to weaning.

Previous evidence indicates that young male rats subjected to a program of very prolonged light exercise (4, 14) or of vigorous exercise of shorter duration (2, 3, 5) gain weight more slowly and have significantly lower final body weights than freely eating sedentary controls. The slower rate of weight gain in response to light exercise appears to be mediated solely by an increase in caloric expenditure as food intake remains constant (4, 14). In contrast, shorter periods of heavy exercise in addition to increasing caloric expenditure also suppress the appetite, resulting in a decrease in caloric intake to account for the lower final body weights of the exercising animals (2). The results of the present study are in keeping with these findings; the rats that swam for 6 hr daily, 6 days/wk (representing very prolonged, light exercise), also gained weight more slowly than sedentary freely eating animals paired with them. This effect of exercise was due solely to an increase in caloric expenditure, as no appetite suppression occurred.

On the basis of the above observations, it would appear that the appetite suppression effect of exercise is related more to the severity of the work than to the duration. Thus, light exercise of long duration such as that performed by the animals in the present study does not suppress the appetite, whereas shorter periods of heavy work do (2). It is our working hypothesis (2) that the appetite suppression is mediated by the increased levels

of catecholamines associated with vigorous exercise (15, 16). In this context, it is of interest that catecholamine levels in the blood are extremely low or close to resting levels during light and moderate exercise but increase markedly in response to heavy work (17, 18). Baile, Zinn, and Mayer (19) reported that food intake can be decreased as a result of intravenous injections of lactate. An alternate hypothesis, then, is that lactate is a mediator of the hypophagia that follows short periods of strenuous exercise, since blood lactate levels can be markedly increased in response to vigorous work (20).

In the past, most of the emphasis has been placed on the correction of the grossly overweight condition, with little attention given to its prevention. To curb the potential for obesity, it appears necessary to control the total number of fat cells which accumulate in the body, since a marked increase in number, rather than an increase in size, is primarily responsible for severe obesity (8).

The results of the present study demonstrate that exercise is effective in reducing the rate of accumulation of cells in epididymal fat pads of rats. If this reduction is a permanent effect of exercise on adipose tissue cellularity, as the work of Hirsch and Han (6) and Johnson et al. (7) suggests, it would have interesting implications with respect to the development of severe obesity. Further studies will be necessary before a broader understanding of the role of exercise in this area is achieved.

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