Longevity of exercising obese hypertensive rats

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BOOTH, F. W., W. F. MacKENZIE, M. J. SEIDER, AND E. W. GOULD. *Longevity of exercising obese hypertensive rats.* J. Appl. Physiol.: Respirat. Environ. Exercise Physiol. 49(4): 634–637, 1980.—The purpose of this study was to determine whether daily running lengthens the life-span of animals dying prematurely due to cardiovascular disease. We used a strain of rat that is genetically hypertensive and obese and is reported to develop atherosclerosis (*Exp. Mol. Pathol.* 19: 53–60, 1973). These animals were divided into three groups consisting of runners exercised daily on treadmills from an early age in life, food-restricted sedentary rats, and ad libitum eaters that were sedentary. This latter group had significantly higher average daily food intakes and body weights than either of the other two groups. The average life-span of both sedentary groups was significantly longer than the running group. Runners had a greater frequency of focal myocardial necrosis, but atherosclerosis was absent in all three groups. We speculate that daily running may have accentuated the development of factors that may have contributed to the early death of runners.

life-span; physical training

PROSPECTIVE STUDIES which test the hypothesis that daily exercise lengthens life expectancy in humans who will develop atherosclerosis are largely absent because of obvious methodological limitations. Animal models are limited by economic requirements. Most species having a short life-span and small body size do not have atherosclerosis as a natural disease. Recently, a new strain (*cp/cp*) of obese hypertensive rats that exhibit many of the risk factors for human atherosclerosis has been described. These factors are hypertension, obesity, and a hyperlipoproteinemia that resembles human type IV (9, 10). In addition this strain was reported to develop spontaneous atherosclerosis and have a premature death (9, 10). This, then, appeared to be an acceptable animal model for a prospective study on the life-extending efficacy of daily exercise. The purpose of the present study was to determine whether daily treadmill running would lengthen the life-span of this strain of rat that dies prematurely of cardiovascular disease.

METHODS

Male and female rats employed in the present studies have the abnormal phenotype of obesity (corpulence) that is inherited as a homozygous recessive trait (*cp*). Animals with obesity (*cp/cp*) cannot produce offspring; corpulent rats were obtained by mating heterozygous nonobese rats (*Cp/cp*). However both the breeders and the corpulent rats were hypertensive. The breeding stock for our studies were descendants of the animals described by Koletsky (9, 10). Koletsky donated some of these animals to the National Institutes of Health who, in turn, provided us with breeders from their colony.

Our animals were housed alone in a room separated from other rodents. All food, bedding, cages, and water bottles were sterilized before being placed in the room. All people entering this room wore masks, gloves, and shoe covers in an attempt to hinder the introduction of exogenous pathogens. These procedures were successful because communicable diseases were not a problem in this study.

The phenotype of obesity becomes apparent between the 5th and 6th wk of life. At this time, obese offspring from our breeders were randomly assigned to one of three experimental groups consisting of ad libitum eaters that were sedentary, ad libitum eaters that were runners, and food-restricted sedentary rats. Each group had 10 animals. Each food-restricted sedentary rat was paired with a runner, and its daily food adjusted so that its body weight equaled the body weight of the runner (Fig. 1). Food intakes were measured daily, and body weights were measured weekly. Rats assigned to the running group began training on a motor-driven treadmill at the age of 45 days. At this time, rats ran 10 min daily. The intensity was progressively increased until the runners were exercising 60 min/day, 7 days/wk at a speed of 20 m/min. They reached this intensity at about 60 days of age. When the rats reached approximately 6–8 mo of age, the intensity of running was reduced to 10–20 min/day at 7 m/min.

All the rats received complete necropsies and histopathologic examinations when moribund or as soon after death as possible. Aortas were collected whole, dehydrated in anhydrous alcohol, and stained with Sudan IV for gross observation of lipid deposition. Frozen sections were also obtained from aortas and stained with Sudan IV. Tissues were processed for paraffin embedding and sectioning by standard methods and then stained with hematoxylin-eosin (13). Special stains were used as needed (13). Sections containing arteries were fixed with glutaraldehyde, postfixed in osmium tetroxide, and embedded in plastic (araldite) also by standard methods (8).

Morphometric analysis of the ventricular myocardium was accomplished by standard quantitative stereoscopic techniques (7). In brief, these procedures consisted of a single microscopic section taken randomly from the long

axis of the heart in such a way as to include right and left ventricular walls as well as the interventricular septum. An ocular reticle with a grid pattern that contains 400 squares with 441 points of line intersection was used. The reticle was randomly placed over 10 areas of the microscopic section of the heart so that a total of 4,500 points per heart were examined. Points overlying abnormal tissue (P_A), which included abnormal muscle, scar tissue, or inflammatory sites, were counted and compared to points overlying normal heart tissue (P_N). The percentage of the ventricular area that was judged abnormal was calculated with the equation, % abnormal area = $P_A/(P_A + P_N) \times 100$ (7).

Systolic blood pressures were indirectly determined from the caudal artery with the tail cuff of a Narco programmed electrosphygmomanometer, as previously described by Tipon et al. (19). Serum triglycerides were measured with the procedure of Bucolo and David (3) and serum cholesterol by the technique of Allain et al. (1).

Results were subjected to statistical analysis with the unpaired Student's *t* test. A 0.05 level was selected as the probability value for significance.

RESULTS

The group of rats that exercised daily lived significantly shorter lives than either of the sedentary groups (Table 1). In confirmation of earlier results (11), food restriction of the sedentary controls resulted in a significantly longer life-span as compared to the ad libitum eaters that were sedentary (Table 1). Body weights of the food-restricted sedentary group and the group of ad libitum eaters that ran were similar and were also significantly less than the body weights of ad libitum eaters that were sedentary (Fig. 1). The average daily food intake was significantly different among all of the three experimental groups (Table 1). Ad libitum eaters that were sedentary ate the most, whereas food-restricted sedentary ate the least.

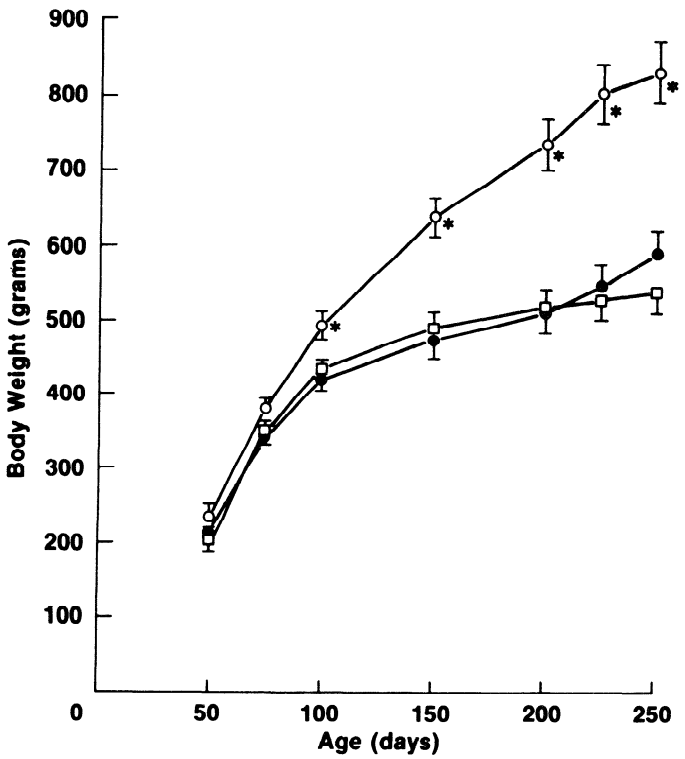

FIG. 1. Body weights of sedentary ad libitum eaters group (○—○), sedentary food-restricted group (□—□), and running ad libitum eaters group (●—●) from 48 to 251 days of age. There are 8–10 rats per mean. * $P < 0.05$ between group of sedentary ad libitum eaters and either the running ad libitum group or the sedentary food-restricted group.

TABLE 1. *Effect of daily running on life-spans, abnormal heart areas, food intake, and caloric intake of obese hypertensive rats*

Measurement	Ad Libitum Eaters, Sedentary	Food-Restricted, Sedentary	Ad Libitum Eaters, Runners
Age of death, days	339.0 ± 22.0*	512.0 ± 28.0*	288.0 ± 7.0*
Percentage of abnormal heart area, %	1.6 ± 0.4	2.8 ± 1.0	7.5 ± 2.1†
Average daily food intake from 48 to 251 days, g/day	35.2 ± 1.3*	21.1 ± 1.1*	30.2 ± 1.0*
Estimated total caloric intake from 48 to 251 days, calories	20,303	12,602	16,426

All values are means ± SE. Number of observations per mean is 10, except for percentage of abnormal heart area where mean is 9–10 rats per group. Caloric intake was calculated using 1 g of food equal to 2.75 kcal. The percentage of abnormal heart area was determined with quantitative stereoscopy as described in METHODS. *$P < 0.05$ among any of the three groups. †$P < 0.05$ between group of running ad libitum eaters and either the sedentary ad libitum eaters or the sedentary food-restricted group.

At 6 mo of age, ad libitum-fed rats that were not members of an experimental group had blood samples taken for lipid analysis. The serum triglyceride value of obese *cp/cp* rats ($n = 3$) was 158 ± 40 mg triglycerides/dl and of nonobese *Cp/cp* rats ($n = 3$) was 56 ± 10 mg/dl. The serum cholesterol value for the obese rats was 240 ± 50 mg cholesterol/dl and 56 ± 8 mg/dl for the nonobese. Although our values of serum cholesterol for the obese rats were higher than previously published values for animals of the same strain and age, our values of serum triglycerides were much lower (10). The values in our animals resembled human type IIb hyperlipoproteinemia (18). After 2 wk of daily acclimatization to procedures, systolic blood pressures averaging 120 ± 8 Torr were measured in 2-mo-old *cp/cp* rats ($n = 5$), whereas values of 142 ± 8 Torr were measured in 3-mo-old *cp/cp* rats ($n = 8$). These measurements are similar to previously published data on rats of the same age and strain (10). At the age of 5 mo, nonobese female rats weighed 251 ± 7 g, whereas obese female rats weighed 631 ± 35 g. Similar findings have previously been published on rats of similar age and strain (10). Massive subcutaneous deposition of fat produced constant contact with the bedding and resulted in decubital ulcers in the posterior abdomen of *cp/cp* rats.

The major difference between the exercised and sedentary animals was the severity of heart disease. Using quantitative stereoscopy, we found that the percentage of the heart area with lesions in the running group was 2.7 times greater than that observed in either sedentary

group (Table 1). The appearance of lesions in hearts was similar in all groups, and they consisted of focal myocardial necrosis and fibrosis.

To obtain additional insight into the cause of death, gross and microscopic evaluation of postmortem tissue was performed by a pathologist. His analyses indicated that heart failure was the cause of death in four runners (one died while running on the treadmill) and that heart failure probably contributed to the death of four other runners. Heart failure was determined as the cause of death in only one rat in each of the sedentary groups. These observations are consistent with our quantitative results (Table 1), showing that runners had the greatest percentage of abnormal heart area. Other observations made on postmortem tissues did not provide enough information to distinguish runners from sedentary groups but did confirm that *cp/cp* rats have multiple disorders. Lesions found in postmortem tissues were severe nephropathy, absence of retinal external lamina, islet of Langerhans hyperplasia, and as yet undefined thyroidopathy. Anemia and hepatic necrosis were occasionally found, and a variety of terminal illnesses were seen in all groups. In contrast to previous reports (9–11) on this strain of rat, atherosclerosis or other vascular disease was not found. Lipid deposition could not be demonstrated in the arteries by a variety of procedures that included gross examination, microscopic examination of Sudan IV-stained sections, and microscopic observation of osmium-fixed, plastic-embedded sections.

DISCUSSION

Daily running significantly reduced life expectancy in the *cp/cp* rat that had numerous disorders that would be called risk factors for atherosclerosis in the human. This animal is obese and hypertensive and has a hyperlipoproteinemia resembling human type IIb (9, 10). The exercise or some facet of the protocol, which was related to the daily running, is probably the factor contributing to their death. Because daily exercise has previously been shown to lengthen the life-span of apparently healthy rodents (4–6, 15, 17), we suggest that the exercise employed in the present study may have been too severe and may have accentuated the disorders in our animal model. The *cp/cp* rats were not in normal health. At necropsy, animals in all three groups usually exhibited multiple endocrine lesions, a protein-losing nephropathy, focal myocardial necrosis, and blindness. In a separate group of *cp/cp* rats, we determined that these disorders were already developing by about 100 days of age. At this age, *cp/cp* rats were also corpulent and hypertensive. Thus the majority of the exercise program was performed with animals that probably had various stages of the disorders mentioned above.

A number of stresses associated with the running program could also have contributed to the earlier deaths of the runners. Because of their extensive inbreeding, *cp/cp* rats were blind. Thus rats in the running group could not see where they were running on the motor-driven treadmill, and this event might have resulted in greater stress for the runners than for the sedentary controls. Another stress that was associated with the running program was the electric shock, which was infrequently used to motivate the rats to run. Sedentary rats did not receive the shock. We were forced to use the exercise model of a motor-driven treatmill with an electric shocker because *cp/cp* rats resisted activity. These rats remained inactive in their cages and did not clean their fur or excretory areas, thus forcing us to clean genital and anal areas to prevent skin irritation. Another possible stress was that this species had a lowered ability to adapt to the daily running. This is supported by the fact that after the runners attained a duration of 1 h of running per day, the runner's exercise tolerance rapidly declined. For the month or so preceding death, runners only walked 10 min/day because they could not endure any greater intensity of exercise. Although it is true that exercise capacity does decline with aging (16), the decrease in exercise tolerance with aging in *cp/cp* runners was very rapid as compared with normal rats (unpublished observations). Because it has been reported that the increase in blood pressure during exercise is greater in hypertensive than in normal patients (12), an increase in blood pressure during exercise in the obese hypertensive rats of the present study could be detrimental to the cardiovascular system. We speculate that any of the aforementioned factors could have further stressed the myocardium of the running group. Previous studies have shown that psychological or catecholamine challenge produced myocardial necrosis in rats (14, 20). To find that the psychological and exercise stresses of the present study exacerbated focal myocardial necrosis in a genetically compromised myocardium is not surprising.

The complete absence of atherosclerosis in the *cp/cp* rat, which was previously claimed to have atherosclerosis, was unexpected (9). The original description of the arterial disease in *cp/cp* rats listed three disease processes: polyarteritis, atherosclerosis, and interstitial deposition of lipid aggregates in the intima and media (9). Similar lesions, lacking lipid deposition, were found in nonobese siblings (*Cp/cp* or *Cp/Cp*) (10). The distribution and description of these previously observed lesions are all similar to the well-described syndrome in the rat thought to be associated with the chronic infectious disease referred to as polyarteritis (2). Polyarteritis is an immune-mediated inflammation of the arteries and is not associated with lipid deposition in the normal rat (2). However, lipid is deposited in all proliferating vascular connective tissue, including venipuncture scars, in other animal models of "atherosclerosis" such as high-lipid, high-cholesterol-fed rabbits that develop severe hyperlipidemia (unpublished observations). If lipid deposition similarly occurred in *cp/cp* rats with polyarteritis, it might then explain the appearance of fat in the arterial lesions referred to as atherosclerosis by the original authors (9, 10). Such rationale might also explain why we observed no atheromatous arterial lesions, since polyarteritis did not occur in the rats of the present study. We speculate that the absence of polyarteritis in our animals was due to the effectiveness of our barrier isolation. Our observations suggest that the *cp/cp* rat is not as good an animal model to study atherosclerosis as originally suggested (9, 10).

It is obvious that an exercise stress on the specific

metabolic disorders of rats in the present study was deleterious. However, because exercise is proving to be beneficial for some other disease states, such as coronary rehabilitation or nonketotic diabetes, our findings demonstrate that we need to learn more about all effects of exercise on various diseases so that exercise prescription can be used judiciously and can be individualized to each patient's disorder.

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