Exercise-Induced Reduction in Myocardial Infarct Size after Coronary Artery Occlusion in the Rat

CARYL L. McELROY, SALLY A. GISSEN, B.S., AND MICHAEL C. FISHEIN, M.D.

SUMMARY Chronic exercise (E) has been thought to be beneficial to the cardiovascular system by increasing energy production and utilization, improving myocardial contractility and increasing myocardial vascularity; whether or not any of these effects are beneficial to ischemic myocardium is uncertain. In this study, rats were forced to swim one hr/day, 5 days/wk for 5 weeks. They were sacrificed and the myocardial capillary bed was perfused with carbon black. Using a calibrated eyepiece grid, histologic sections of heart were examined to determine capillary/fiber ratios (C/F) and myocardial fiber diameter. C/F was increased by 30% in E rats (0.87 ± 0.06 (mean ± SE) (N = 4 rats)] when compared with sedentary controls (C) [0.67 ± 0.04 (N = 4 rats), P < 0.05]. This training increases in the size of the coronary tree, anastomotic development, capillary/fiber ratios, and luminal cross-sectional area of coronary vessels that have been demonstrated in animals subjected to exercise programs may augment oxygen and substrate delivery to myocardium.

There is evidence that the hearts of conditioned animals respond more effectively to stress. Trained rats, when subjected to sustained (1-3 days) pressure overload by aortic constriction, are able to maintain or increase myocardial contractility while sedentary animals cannot. Under hypoxic conditions exercised rats maintained higher levels of contractility, ventricular pressure, and heart rate.

The potentially beneficial effects of chronic exercise and their mechanisms of action usually have been studied in healthy experimental animals, rather than animals subjected to myocardial ischemia. Therefore, there is little direct evidence in man or experimental animals that these effects would protect ischemic myocardium which should benefit most from the cardiovascular adaptations to exercise. This study was undertaken to determine whether chronic exercise could protect ischemic myocardium and reduce myocardial infarct size after coronary artery occlusion in rats.

Methods

I. Training Effects of Chronic Exercise

A study was first conducted to determine whether training effects could be demonstrated in rats subjected to an exercise program that consisted of a swim session five days a week for a given number of weeks. Sixteen three-week-old male albino rats of the Sprague-Dawley strain (Charles River Breeding Laboratories) were divided into two groups: eight sedentary controls and eight exercised rats. The animals were housed in groups of four in 45 × 25 × 20 cm cages. Food (Purina Laboratory Chow) and water were provided ad libitum. Food consumption and each rat's body weight were measured three times weekly.

The exercised rats swam in a 50 (height) × 35 (diameter) cm cylindrical container. Water depth was 35 cm and temperature was maintained between 35° and 37° C. The training program was initiated with a 35-minute swim which was extended to 60 minutes by increasing the swim time by five minutes each day. After swimming, the rats were towel-
dried and returned to their cages. In order to separate the effects of exercise from those due to handling (immersion in water and towel-drying) the control rats were placed in 37°C water that was shallow enough to permit them to stand (rather than swim) for a 60-second period. Then, like the exercised rats, they were towel-dried and returned to their cages.

Half of the rats (four controls and four exercised) were sacrificed after five weeks of swimming, the remaining after six weeks. At the time of sacrifice each rat was injected with heparin sodium (100 units) which was allowed to circulate for 10 minutes. Following ether anesthesia, the heart was excised along with the ascending aorta. The aorta was cannulated and the heart flushed retrograde with saline at a pressure of 90 mm Hg for two minutes to remove blood. The heart was fixed by perfusion with 10% phosphate-buffered formalin at the same pressure for 10 minutes. Finally, black ink (Pelikan Special Ink, C11/1431 A) was infused for one minute for demonstration of the capillary bed. This was followed by immersion of the heart in 10% formalin for 24 hours. The aorta and atria were dissected away from the heart and the ventricular weight was measured prior to routine processing for histologic examination.

To determine capillary/fiber ratios, histologic sections were examined at a magnification of 400X. A 5 mm x 5 mm calibrated eyepiece grid was used to delineate areas used for counting. All counts were done on fields in which fibers and capillaries appeared round (rather than cylindrical or ellipsoidal) indicating that they were cut perfectly transversely. Thus, only true cross-sections were counted. The percentage of the left ventricle composed of capillaries was estimated by point-counting techniques.\textsuperscript{18} The justification for the use of systematic sampling of cross-sections, rather than random sampling, has been published in detail by Weibel.\textsuperscript{18} Two hundred points were counted from each rat heart. Fiber diameters were measured at a magnification of 1000X using the calibrated eyepiece grid.

II. Effects of Exercise on Infarct Size

Having established through the first study that several training effects (see Results) can be observed in rats that have swum one hour a day, five days a week for five weeks, three-week-old male rats were subjected to the training program as described above with the exception that a 264-liter, 36 x 50 x 120 cm glass tank was utilized. The rats that served as controls were treated in the same manner as the control group in the first study.

Twenty-four hours after the termination of the training program, a total of 90 exercised and control rats underwent surgical occlusion of the left coronary artery by technique described in detail elsewhere.\textsuperscript{19,20} In brief, each rat was anesthetized with ether and after a 2-cm incision had been made along the left sternal border, the 5th and 6th ribs were separated and the heart exteriorized by compression of the lateral aspects of the thoracic cage. With a 4-0 silk suture the left coronary artery was occluded 3-4 mm from its origin. The chests were closed and the rats allowed to recover. Operative mortality, which was similar in both groups, was 42% and was related to excessive anesthesia, intraoperative technical difficulties and pulmonary edema in rats with very large infarcts which were incompatible with survival.

Forty-eight hours after coronary artery occlusion, when myocardial necrosis is at its peak, the rats were reanesthetized and sacrificed by excision of the heart. The hearts were fixed in 10% phosphate-buffered formalin for 24 hours. The ventricles were weighed, then sectioned from apex to base in a plane parallel to the atrioventricular groove. Four slices measuring 2 to 2.5 mm in thickness were obtained from each heart. Following dehydration, clearing and embedding all four of the tissue slices from each heart were sectioned and mounted on 2" x 2" glass slides and stained with hematoxylin-eosin.

To quantify infarct size, histologic sections of the four slices of each heart were projected onto a screen at a magnification of 10X and a planimeter was used to measure the areas of infarcted and noninfarcted left ventricular myocardium. From these measurements the percent, by area, of the left ventricle which was infarcted was calculated. This percentage represents an estimate of the percent of the total volume of the left ventricular myocardium which was infarcted. The mathematical justification for using 2-dimensional tissue sections to make quantitative estimates of 3-dimensional structures (stereology) has been described in detail elsewhere.\textsuperscript{18} Student's t-test (unpaired) was used to compare findings in the exercised and control rats.

### Results

#### I. Training Effects of Chronic Exercise

The various parameters analyzed in the first study in rats subjected to five weeks of exercise are summarized in table 1. The weight gain of the exercised rats was 9% less ($P < 0.005$) than that of the sedentary controls despite the fact that the exercised rats ate similar amounts of food. Ventricular weights and myocardial fiber diameters were similar in the two groups; thus, there was no cardiac hypertrophy in

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<th>Table 1. Training Effects of Chronic Exercise</th>
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<td><strong>Initial body weight (g)</strong></td>
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<td><strong>Final body weight (g) (after the exercise period)</strong></td>
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<td><strong>Weekly food intake (g)</strong></td>
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<td><strong>Ventricular weight (mg)</strong></td>
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<td><strong>Myocardial fiber diameter (microns)</strong></td>
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<td><strong>Capillary/Fiber ratio</strong></td>
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<td><strong>Area of capillary bed (% of left ventricle)</strong></td>
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*Mean ± ss.
the exercised rats. Significant increases in the mean capillary/fiber ratio (0.87 ± 0.06 vs 0.67 ± 0.04, P < 0.05) and relative area of the capillary bed in the left ventricle (28.8 ± 0.5% vs 23.6 ± 0.6%, P < 0.001) were found in the exercised rats when compared with controls.

II. Effects of Exercise on Infarct Size

The results of the infarct study are shown in table 2. As in the first study the growth rate of the swimming rats was slower than that of the controls. Ventricular weights were equivalent in the two groups. In the 27 rats in the exercise group the infarct size was 21.5 ± 1.9% of the left ventricle as compared with 31.3 ± 2.6% (P < 0.005) in the 25 sedentary control rats (fig. 1). The difference in infarct size between the two groups represents a 30% decrease in infarct size in the exercised animals.

Discussion

In this study young male rats were chronically exercised by a swimming program consisting of one hour of swimming per day, five days per week for five weeks. An initial study showed that this program produced training effects. When compared with controls, exercised rats weighed 9% less, even though food consumption was similar, and had a 30% increase in capillary/fiber ratio. Acute ischemia was induced in larger groups of exercised and sedentary rats by ligation of the left coronary artery and the size of the subsequent infarction was measured to determine whether chronic exercise would be associated with preservation of myocardium. A 30% decrease in infarct size was observed in the chronically exercised rats.

In this study, attempts were made to ensure that the results obtained could be attributed solely to exercise. The swimming regimen was designed to eliminate (as much as possible) stress as a variable. The stress that may have been involved in handling the rats before and after the swim session and in immersing them in water was duplicated in the controls. Swimming rats were introduced to the training program gradually to allow time for acclimatization. Rats were swum in thermoneutral water (37°C) which is associated with stabilized heart rates and body temperatures in rats and believed, for these reasons, to be submaximal exercise. Examination of the hearts at the termination of the exercise program revealed no gross or microscopic evidence of cardiac hypertrophy. In addition, there were no areas of fibrosis or necrosis, which are also associated with more strenuous and stressful regimens.

In man, weight loss is a well-known and desirable effect of physical conditioning. In young rats, retardation of the growth rate with exercise has been observed in many studies involving training regimens. In our study, as in a study by Oscai et al., the exercised rats gained less weight than controls in spite of similar food intake. This retardation of growth cannot be interpreted as being due mainly to stress-induced appetite suppression, as has been suggested, but

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<th>Table 2. Effect of Exercise on Infarct Size</th>
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<td>Control (25 rats)</td>
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<tr>
<td>Initial body weight (g)</td>
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<td>Final body weight (g) (after the exercise period)</td>
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<td>Ventricular weight (mg)</td>
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<td>Myocardial infarct size (% of left ventricle)</td>
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Figure 1. Histologic sections of serial transverse slices of heart from examples of control (A) and exercise (B) rats sacrificed 48 hr after coronary artery occlusion. The infarct (i) (between broken lines) in this control rat (A) involved 43.4% of the left ventricle (LV). The infarct (i) in the exercised rat (B) involved 21.1% of the left ventricle. RV = right ventricle. Hematoxylin-eosin stain, ×7.
rather to increased caloric expenditure associated with vigorous physical activity.

Myocardial vascular changes have also been interpreted as being adaptations to chronic exercise. Increased vascularization per unit mass of myocardium, enlargement of epicardial coronary arteries, enlargement of extracardiac sources of collateral vessels and increased myocardial capillary/fiber ratios have all been demonstrated after chronic exercise. In our study a 30% increase in capillary/fiber ratios was observed in the exercised rats. This increase is similar in magnitude to that reported by Tittel et al. and Leon and Bloor but greater than that reported by Tomanek as he observed less than a 10% increase in exercised rats. This difference, and differences in absolute capillary/fiber ratios observed by different investigators, may be due to several factors including the age and strain of rat, the training program employed, and the methodology involved in injecting and counting capillaries. The increased vascularization associated with exercise does not appear to be a result of severe hypoxia since it has been observed in animals subjected to only moderate, or even intermittent, exercise programs. In our study, the increase in capillary/fiber ratio occurred without morphologic evidence of severe myocardial hypoxia, since there was no necrosis or fibrosis and no myocardial hypertrophy.

There is some evidence that collaterals, such as those induced by exercise, are of functional significance. In studies by Bloor et al., the presence of collaterals was shown to favorably affect the fibrillation threshold after acute coronary artery occlusion. Dogs with better collaterals had a higher fibrillation threshold and had less of a rise in serum CK activity following coronary occlusion, suggesting that they had smaller infarcts than dogs without collaterals. Schaper and Pasyk, using microsphere techniques, have shown that in the dog the time interval between the onset of ischemia and the no-reflow phenomenon depends on the amount of collateral flow. Since, in that study, the no-reflow phenomenon occurred at about the same time as irreversible cell death, the authors concluded that myocardial salvage may be possible so long as perfusion can be maintained through collaterals. Thus, these studies indicate that there are beneficial effects of collaterals which have the potential to preserve ischemic myocardium.

While the results of this study may appear encouraging to those of us who exercise, they must be interpreted with caution. Acute coronary artery occlusion in the rat with normal coronary arteries is different from slowly progressive atherosclerotic occlusion of the coronary arteries in man. In addition, in spite of our efforts, it is not possible to be certain that our two groups of rats differed only in their level of physical activity and that some other factors may be responsible for the observed differences in infarct size. However, this rat model has been used to study the effects of a number of other interventions on infarct size. Several pharmacologic agents which have reduced infarct size in the dog have also been found to be beneficial in rats. One drug, hyaluronidase, which has been shown to reduce infarct size in the rat, has recently been found to be beneficial in man as well. Also, in this rat model, it has been shown that the sparing effect of several drug interventions which is evident at 48 hours is also observed at 21 days when the healing process is complete. Thus, the exercise-induced salvage of myocardium we observed at 48 hours should persist and result in permanent preservation of myocardium when scar formation is complete.

This study demonstrates that chronically exercised rats, in which training effects have been produced, will have smaller infarcts than sedentary control rats when both are subjected to acute coronary occlusion. On the basis of previous studies, it would seem that the increase in myocardial vascular which is a demonstrated effect of chronic exercise may play an important role in the preservation of myocardium subjected to acute ischemia. We cannot, however, discount the possible role of other anatomic, hemodynamic, metabolic and neurohumoral alterations associated with regular physical activity.

Acknowledgment

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Effects of Acute Cellular Injury on Coronary Vascular Reactivity in Awake Dogs

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SUMMARY The study was designed to examine effects of acute cellular injury on regional myocardial blood flow (RMBF) and coronary vascular reactivity. Before myocardial infarction in 14 dogs, RMBF was measured using 7–10μm microspheres during the hyperemic response following a 60 sec transient ischemic stimulation (TIS). Myocardial infarction was induced by complete occlusion for two hours and then inflow to the injured area was re-established. RMBF was measured four hours later during basal conditions, following a 60 sec TIS and during infusion of adenosine, 1.0 mg/kg/min. Effects of acute cellular injury were examined by measuring RMBF in multiple myocardial samples, grouped according to extent of histologic necrosis.

Four hours after reperfusion, RMBF was decreased when infarction exceeded 50%. The decrements in flow were directly proportional to the extent of infarction. The vasculature was capable of delivering additional flow to the injured area since both TIS and adenosine infusion affected increases in RMBF in excess of 100% in each region of the ischemic zone. Blood flow responses to these stimuli, however, fell in proportion to the extent of infarction. RMBF responses to TIS and adenosine infusion were comparable, indicating ischemia which effects irreversible myocardial injury also directly alters vasomotor properties of the intramural vasculature.

ISCHEMIA OF THE MYOCARDIUM sufficient to initiate acute cellular injury affects local responses in the zone of ischemia which directly alter tissue perfusion. Studies from this laboratory have demonstrated that the alterations in regional myocardial perfusion which result from acute cellular injury are directly proportional to the extent of eventual histologic myocardial necrosis.

The objectives of the present study were to determine 1) whether factors which effect reduction in blood flow to an acutely injured area also prevent increases in blood flow in response to the metabolic stimuli resulting from transient ischemia, and 2) whether ischemia sufficient to effect irreversible myocardial injury also directly alters the vasomotor properties of the intramural vasculature. Regional myocardial blood flow was measured after re-establishing inflow to an area subjected to two hours of ischemia. Regional myocardial blood flow was measured during basal conditions, immediately following the metabolic stimulation resulting from transient ischemia, and during direct vascular stimulation effected by intravenous adenosine. Effects of acute cellular injury on myocardial perfusion during these interventions were assessed by determining regional blood flow in multiple myocardial samples grouped according to subsequent histologic myocardial infarction. The study was performed in awake, chronically prepared animals to avoid variables introduced by general anesthesia and acute surgery.

Methods

Complete studies were performed in 14 mongrel dogs weighing 25–34 kg. The dogs were anesthetized with thiopental sodium (30–40 mg/kg, i.v.) and underwent a left thoracotomy. The proximal 1 cm of the left circumflex coronary artery was dissected free and a pneumatic cuff occluder was placed around the vessel. Heparin-filled catheters were inserted into the left atrial cavity and the aortic root. The catheters and snare were tunneled to a subcutaneous pouch at the base of the neck.

Studies were performed 7–10 days after the surgical procedure with the dogs lying quietly on a laboratory table as previously described. The mean hematocrit at the time of study was 42%, range 38–52%. To assure proper function of...
Exercise-induced reduction in myocardial infarct size after coronary artery occlusion in the rat.
C L McElroy, S A Gissen and M C Fishbein

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