THE UNIVERSITY OF MANITOBA

THE EFFECTS OF EXERCISE STRESS ON THE RAT HEART

BY
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A Thesis
Submitted to the Faculty of Graduate Studies
in Partial Fulfillment of the Requirements for
the Degree of Master of Science.

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To My Family

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ABSTRACT

Although it is generally believed that exercise is beneficial for an individual, experimental data in support of this view is less than conclusive. In this regard, we have studied two important aspects of the effects of chronic exercise on the heart 1) Is exercise good for the peart per se? 2) Does chronic exercise improve the capability of the heart to withstand a subsequent stress? For this purpose rats were subjected to an exhaustive four week swimming programme, twice daily, five days a week at a water temperature of 22° C. The sedentary rats were maintained at normal cage activities. At the end of four weeks most of the exercise stressed as well as sedentary rats were injected with different concentrations of isoproterenol (20,40 or 80 mg/kg) and the remainder of the animals not injected with isoproterenol served as controls. Electrocardiography (EKG), serum enzyme analysis and heart histology were performed on all rats to assess the beneficial and/or detrimental effects of chronic exercise.

At sacrifice, the exercise stressed control animals had significantly lower body weights, increased wet heart weights, and increased heart weight/body weight ratios as compared to the sedentary rats. Furthermore, these untreated control rats exhibited a significant increase in the amplitude of the R wave. Although heart rate (HR) was slightly decreased in the exercised rats, this was not significant. Serum glutamic oxaloacetic transaminase (SGOT) was elevated in the exercise control group in the absence of any alteration in lactate dehydrogenase (LDH) and creatine phosphokinase (CPK). Focal necroses and increased collagen formation was apparent in hearts from exercise

stressed controls on light microscopic examination.

Following injection with isoproterenol, the exercise stressed rats showed no increase in heart weight/body weight ratios in contrast to significant increases in the sedentary animals treated with isoproterenol. The exercised treated animals exhibited a significantly greater tachycardia response to isoproterenol within 15 minutes of the drug administration. Subsequently, the EKG's showed progressive slowing of heart rate and conduction defects in exercised rats. In contrast, some of the sedentary treated rats demonstrated ventricular arrhythmias progressing to ventricular fibrillation. Mortality was significantly greater in the exercise stressed group but appeared to be associated with symptoms of pulmonary edema. Serum CPK, LDH and SGOT were all significantly elevated in sedentary and exercise stressed rats injected with 80 mg/kg isoproterenol. However, SGOT was significantly higher at all doses of isoproterenol in exercise stressed rats. At all doses of isoproterenol, hearts from sedentary treated rats demonstrated greater histological damage than did hearts from exercise stressed animals.

These data indicated that the stressful exercise protocol induced myocardial necrosis in the control rats which appears to have produced an adaptation that protected the myocardium from further damage when the exercised rats were exposed to another stress (isoproterenol). Thus, exercise appears to be detrimental as well as beneficial to the heart. Further research into the intensity and type of exercise is required to define the limits which will result in only beneficial effects.

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I INTRODUCTION

Acute as well as chronic exercise protocols are a form of stress. Acute exercise induces many of the same cardiovascular reponses attributed to other forms of stress such as heat, cold or psychological factors. Chronic exercise,on the other hand, induces cardiovascular compensatory adaptations which may include increased sympathetic tone and heart hypertrophy. Whether these adaptive changes in response to chronic exercise are detrimental or beneficial remains to be elucidated. Furthermore, how these adaptive effects in response to chronic exercise can be modified if this stress is combined with some other stress is also not known.

The effects of chronic exercise have been the subject of a great many studies in both human and animal models. The beneficial effects are generally attributed to an improved pumping efficiency of the heart through an increased stroke volume and a lower resting heart rate, and through peripheral cardiovascular adaptations. These include enhancement of skeletal muscle metabolism and performance and an enhanced skeletal muscle oxygen extraction.

In animal models, both beneficial and detrimental effects of exercise on the heart have been observed. Generally, the detrimental effects have been observed in exhaustive exercise programmes or in programmes where the exercise was associated with other stresses, such as intense heat or cold. In exercise protocols where other forms of stress are minimized, the adaptation in cardiac performance has been considered beneficial.

There has been a paucity of work examining the combined effects of exercise and other stressors. The literature that is available suggests that exercise combined with other stressful influences, in contrast to exercise alone, may be detrimental to cardiac performance.

The purpose of this investigation was to examine the effects of exercise stress on cardiac performance.

An exhaustive swimming exercise protocol was employed. The cardiovascular response of the exercised rats in comparison to sedentary animals was monitored by employing electrocardiography, serum enzyme analysis and histological examination. The response of the exercise stressed rats to various doses of isoproterenol was also studied.

II LITERATURE REVIEW

THE EFFECTS OF CHRONIC EXERCISE ON THE HEART

A) GENERAL

Exercise training produces profound biochemical, morphological and functional changes in the cardiovascular system. The effects of chronic exercise on the autonomic nervous system, on myocardial structure and function, and the responses to hypoxia and ischemia will be reviewed.

The animal models which have been most extensively employed to study these effects are the rat and the dog. Exercise studies utilize two major modalities, treadmill running (Krames et al,1976; Barnard et al, 1971; Maher et al, 1972; Sordahl et al,1977; Dowell, et al, 1977; Carew et al, 1978; Schaikle et al, 1979) or swimming, (Baker et al, 1964; Dawson et al, 1968; Penpargkul et al, 1970; Wilkerson et al, 1971; Bhan et al, 1972; Scheuer et al,1974; Bhan et al, 1975; Holloszy et al, 1976; Bershon et al, 1977; Penpargkul et al, 1977; Penpargkul et al, 1978). There are several problems in comparing the results obtained in different species and different exercise models where duration, intensity and the frequency of training protocols vary. The effects of swimming protocols will be reviewed. Reference to treadmill findings will be presented to further support or to refute the effects found in swim trained animals.

B) EFFECTS ON AUTONOMIC NERVOUS SYSTEM

Miyagi et al (1979) observed that the stress of exercise and the stress of hypoxemia both equally induced sympathetic nervous system excitation. They measured catecholamine levels in the coronary

sinus and arterial plasma in man and found no significant differences between either stressor. Scheuer and Tipton (1977) referred to exercise as a "disruption of a homeostatic relationship that has been caused by bodily movement". The cardiovascular effects resulting from a stress activated sympthetic response are the same regardless of the type of stress but the metabolic effects are specific to the stress situation (Leblanc, 1969).

Increased central nervous system (CNS) resting norepinephrine levels have been observed in exercise trained rats (Brown and Van Huss, 1973). If norepinephrine modulates central sympathetic tone this may represent a higher maintained level of sympathetic output in conditioned animals as compared to sedentary animals.

Brown et al (1973) further observed that exercise training evoked a greater depletion of brain norepinephrine than shock stress to sedentary rats and suggested that exercise evoked a greater sympathetic discharge than shock stress.

DeSchryver et al (1972) reported that chronic exercise induces a decrease in the sympathetic neurotransmitter, norepinephrine, in cardiac tissue. They also observed a close relationship between the intensity of exercise and cardiac catecholamine depletion and found that there was no difference in myocardial norepinephrine levels in animals trained by either spontaneous or forced running (DeSchryver et al, 1972). In other studies, DeSchryver et al (1968) observed that the catecholamine response is reversible and that on cessation of training, normal levels are restored within six days.

Crews et al (1967) and Maher et al (1972) found that cardiac endogenous catecholamine stores were unchanged following

exercise. Crews et al (1967) swim trained rats and then studied cardiac function in an open chest in vivo preparation. Intravenous injection of 3 mg/kg of epinephrine elicited a decrease in isometric systolic tension development of the right ventricle. Similarly, Maher et al (1972) observed a negative inotropic effect when isolated trabeculae muscle from treadmill exercised rats was exposed to a bath containing 0.3 mg/ml norepinephrine. Crews et al (1967) suggested this hyposensitivity may reflect a relative refractoriness of the myocardium to increased circulating catecholamines during muscular exercise and that this decreased sensitivity to catecholamines deprives the heart of an important compensatory mechanism which the sympathetic nervous system usually provides.

In contrast to the report of Crews and co-workers (1967) Wyatt et al (1978) observed that training enhances the sensitivity of cardiac muscle to catecholamines. They suggest the results of Crews et al (1967) may have been affected by the anesthesia and thoracotomy for open chest in vivo observations. Wyatt et al (1978) found an increased adenylate cyclase activity following isoproterenol administration in trained cats. He suggested the affinity of adenylate cyclase for isoproterenol was unchanged by training but that the maximum velocity of interaction was greater. Dowell and Tipton (1970) found that hearts from trained rats beat faster after infusion or injection of isoproterenol than hearts from non-trained animals.

Salzman et al (1970) exercise trained mice and found that the ventricles of these mice were more sensitive to high doses of exogenous epinephrine than sedentary controls and that males were

more sensitive than females. However, the uptake of catecholamines by the sympathetic nerve endings in the myocardium was decreased. The decreased uptake of catecholamines suggests that more of the catecholamines may be free to act on other receptor sites and exert their influence on the heart (Salzman et al,1970).

Resting bradycardia is a widely accepted result of physical training programmes in animal models (Lin and Horvath,1972;Carey and Tipton,1976; Hughson et al,1977; Dowell et al,1977;Schaible et al 1979). The exact mechanism for the development of bradycardia in conditioned animals is unclear. However, current evidence clearly indicates that an alteration in autonomic nervous system activity plays a significant role.

Hughson et al (1977) found that rats maintained on treadmill exercise exhibited decreased intrinsic sino-atrial rate and that this phenomenon occurred independently of any increased parasympathetic activity. Atropine did not block the decreased intrinsic activity in exercised rats but rather enhanced it. These authors further noted that the chronotropic effects of norepinephrine on isolated atria removed from exercised rats were significantly attenuated although the response to acetylcholine was unaltered.

Lin and Horvath (1972) swim trained rats to produce a bradycardia response. They found a greater decrease in sympathetic tone than parasympathetic tone following training, but also observed that both sympathetic and parasympathetic activity was decreased in exercised animals as compared to sedentary rats. Bolter et al (1973) in swim trained rats found a marked subsensitivity to acetylcholine and concluded that this represented an increased tonic vagal cardiac inhibitory activity. He also noted that with total autonomic blockade

with atropine and propranolol, isolated atrial preparations removed from trained animals had significantly lower spontaneous rates than atria removed from untrained animals.

C) MYOCARDIAL HYPERTROPHY

Myocardial hypertrophy is another frequently observed result of an exercise training programme (Krames, et al, 1967; Leon and Bloor, 1968; Penpargkul et al, 1970; Wilkerson et al, 1971; Bhan et al,1972; Scheuer et al, 1974; Carew and Covell, 1978; Hickson et al,1979). However, some authors have found no increase in ventricular mass in dogs trained by treadmill running (Sordhal et al, 1977; Dowell et al, 1977). They further reported that in the absence of ventricular hypertrophy the maximal rate of ventricular pressure development was increased. Tibbits et al, (1978), also found no significant myocardial hypertrophy in rats trained on a treadmill. They further suggested that swimming produces hypertrophy because it places a more significant stress on the heart.

In contrast, Carew et al (1978) observed significant left ventricular hypertrophy in exercise trained greyhounds, but no change in resting contractility or in contractility indices at increased end-diastolic pressures as compared to normal dogs. Rats trained on the treadmill have also been shown to develop myocardial hypertrophy (Krames et al, 1967). However, they measured isometric pressure development of the left ventricle in vivo and did not find any differences in maximum pressures between exercised and control animals. Scheuer and Tipton (1977) stated that forced running resulted in a greater increase in heart weight than spontaneous running. Increased heart weights have been observed repeatedly in swim trained

rats (Bhan et al, 1972; Scheuer et al, 1974; Scheuer and Tipton, 1977).

Leon and Bloor (1968) found that myocardial hypertrophy occurred in continuously exercised rats, but not in animals on an intermittent training programme.

Hypertrophy is considered to be a compensatory mechanism resulting from a sustained period of hyperfunction (Meerson,1974).It has been suggested that exercise protocols of moderate intensity do not produce myocardial hypertrophy whereas more stressful exercise programmes do induce hypertrophy (Bershon et al, 1977).

In view of the contradictory reports regarding the development of myocardial hypertrophy induced by exercise training, an increase in muscle mass should not be considered a sensitive index of training responses in animals.

When myocardial hypertrophy is observed in animal models, it involves an increase in collagen formation as well as an enlargement of myofibrils (Grove et al,1969;Bartosova et al, 1969;Skosey et al, 1972; Zak, 1974; Morkin et al, 1974; Meerson et al, 1974). The contribution of each to the increase in mass is variable depending partially at least on the type of stimulus inducing hypertrophy, the length of its action (Bartosova et al, 1959) and the age of the animal (Bartosova et al, 1969; Zak,1974). Bartosova et al (1969) observed a marked increase in wet heart weight and in collagen formation in two and a half month old rats subjected to a six week treadmill exercise programme. However, they found no increase in either wet heart weight or collagen formation in eight and a half month old rats trained on the same programme. Hypoxia is a powerful stimulus to fibroblast activity and

increased collagen formation in the heart (Bartosova et al, 1969). Zak (1974) reported that in adult rats under work overload only hyperplasia of the connective tissue cells occurred and that in growing animals hyperplasia of both myocytes and connective tissue cells occurred. In adult rats existing myocytes increased in volume not in number (Grove et al, 1969; Carew et al, 1978).

Meerson et al (1974) (1978) stated that hypertrophy of the myocardium as an adaptive mechanism is relative, and that a long period of extensive hypertrophy is usually followed by a more rapid aging of the myocardium.

D) SUBCELLULAR CHANGES

Leon and Bloor (1968) exercise trained rats by swimming in 28-32^{o} C water. They observed a significant increase in coronary artery diameter with training. As well, they observed focal necroses in the myocardium of animals subjected to a six day a week programme, whereas, intermittently exercised rats did not exhibit myocardial lesions. They suggested that a relative hypoxia due to the increased metabolic demands during exercise served as the stimulus for the developed vascular changes and that the focal necroses resulted from a relative coronary insufficiency. The lesions consisted of hyalization of muscle fibers, loss of myofibril bundles, and an extensive mononuclear cellular infiltrate indicating that the lesion was in the later phases of healing.

It is generally accepted that cardiac mitochondria have the reserve capacity to meet the energy demands of most exercise training protocols without compensatory adaptations. No changes in mitochondrial cytochrome C oxidase, ATPase activity or calcium content

has been observed in swim trained rats (Penpargkul et al,1978). Dohnm et al (1972) also found no difference in heart mitochondrial oxygen uptake in treadmill trained rats. Sordhal et al (1977) and Dowell et al (1977) reported that unlike skeletal muscle, the heart does not undergo an adpative increase in respiratory capacity as a result of exercise training. They found the activities of mitochondrial enzymes, the concentration of cytochrome C oxidase and the protein expressed per gram of heart were unchanged following training.

In contrast to more moderate exercise training, exhaustive exercise has demonstrated swelling and disruption of cardiac mitochondria (King et al, 1970; Banister et al,1971; Dohm et al, 1972). The adverse effects disappear approximately twenty-four hours after the exhaustive exercise session (Banister et al, 1971). In contradiction to these findings, Terjung et al (1973) observed that a single bout of exhaustive exercise employing both swimming and treadmill modalities, resulted in no change in the respiratory capacities or citrate synthase activity of the heart mitochondria. He also found normal mitochondrial yields and normal appearance of mitochondria on electon microscopic examination.

E) EXERCISE TRAINING AND RESPONSES TO HYPOXIA AND ISCHEMIA

Carey and Tipton (1976) found that treadmill trained rats were able to maintain a higher level of cardiac performance (dP/dt max) following hypoxia. They suggested that training may increase receptor sensitivity to released and/or circulating catecholamines, and that this may be related to the improved performance of trained animals during hypoxia. Scheuer et al (1972) found that hearts from rats conditioned by swimming have enhanced pumping ability when subjected to hypoxia.

They observed that conditioned hearts develop the same systolic pressure from a lower end diastolic pressure than hearts from sedentary rats. However, under ischemic conditions trained animals demonstrated a more rapid decline in myocardial performance than sedentary animals (Carey and Tipton,1976).

F) SWIMMING EXERCISE

1) Swimming Exercise at Thermoneutrality

The swimming protocols designed to investigate only exercise responses involved swimming rats at water temperatures between 33-37° C. Extensive research has been conducted utilizing this exercise model. Bershon and Scheuer (1977) swim trained rats for eight weeks and then measured left ventricular and aortic pressures, and cardiac output in an *in vitro* isolated working heart model. From these measurements, they approximated values for external work. They demonstrated an increased cardiac performance in exercised rats as compared to sedentary animals at the same end diastolic pressure and volume, and at all atrial pressures recorded between 5 and 20 cm H_2O. They further recorded an increased ejection fraction, peak systolic pressure, peak aortic flow, cardiac output and stroke work in conditioned hearts. It was concluded that the enhanced pumping performance was due to a change in ventricular muscle function. Faster relaxation was a prominent effect of physical training (Bershon et al, 1977; Penpargkul et al, 1978). This improved relaxation may be related to the greater calcium uptake and binding in the sarcoplasmic reticulum (SR) of exercise trained rats (Penpargkul et al,1977). Penpargkul et al, (1977) observed that cardiac

microsomes from conditioned hearts transport calcium to a greater extent than microsomes from sedentary rats. In contrast, Sordhal et al (1977) found no differences in rates of calcium uptake or binding by the SR in treadmill trained dogs. They also observed a decreased rate of release of bound calcium from the SR .

Exhaustive exercise in rats has been found to depress calcium uptake by the SR by approximately 50 percent and to lower calcium stimulated ATPase activities (Sembrowich et al, 1977; and Hashimoto et al, 1978). Maher et al (1972) also found exhaustive treadmill exercise in rats produced a marked depression of myocardial performance.

Interestingly, Sordhal et al (1977) observed no significant difference in mitochondrial calcium uptake in trained dogs. However, he found that the mitochondria from trained dogs failed to take up all the calcium present, and released calcium more rapidly than did control animals.

Other investigations by Bhan and Scheuer (1972 and 1975) and Wilkerson et al (1971) have found increased actomyosin and myosin adenosine triphosphatase (ATPase) activities in conditioned hearts. The improved contractility of the left ventricle may be related to alterations in calcium transport mechanisms or to improved intrinsic functioning of the contractile proteins (Bahn et al, 1972). Crews et al (1967) also found increased myocardial contractility in exercise trained rats. Tibbits et al (1978) in Lanthanum studies on isolated papillary muscle from treadmill trained rats suggest

an enhanced extracellular calcium availability in trained animals and suggested that this increased calcium may play a role in the improved myocardial performance.

Schaible et al (1979) found that the increased rates of relaxation and the increased actomyosin ATPase activities which occurred in swim trained rats did not occur in treadmill trained animals. Dowell et al (1977) also found normal ATPase activities in treadmill trained dogs who demonstrated an increased myocardial contractile performance. This suggests possible differences in the biochemical adaptations between swimming and treadmill training protocols.

It appears indesputable that alterations in calcium transport occur as a result of exercise training. However, the precise mechanism or mechanisms and the contribution of each to enhanced or diminished performance remains unclear.

Furthermore, a greater increase in oxygen consumption, cardiac output and cardiac work has been osberved in conditioned hearts subjected to increasing atrial filling pressures compared to hearts from sedentary rats (Penpargkul et al,1970). The improved dynamic responses were considered to be due, at least in part, to the improved coronary blood flow in trained hearts allowing enhanced mechanisms of oxygen delivery.

2) Swimming Exercise and Hypothermic Stress

Water temperature is critical when employing swimming as the exercise modality. The temperature must be maintained between 33^{o} C and 37^{o} C to obtain a thermoneutral environment