THE EFFECTS OF ENDURANCE EXERCISE TRAINING ON THE CORONARY VASCULAR RESPONSIVENESS TO INTRACORONARY ACETYLCHOLINE IN SWINE

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ABSTRACT

Title of Dissertation: The Effects of Endurance Exercise Training on the Coronary Vascular Responsiveness to Intracoronary Acetylcholine in Swine

Brenda A. Tondi, Doctor of Philosophy, 1993

Dissertation Directed by: Jack E. McKenzie, Ph.D., Associate Professor; Department of Physiology

Acetylcholine, the parasympathetic nervous system neurotransmitter, is a potent vasoconstrictor of the coronary vasculature in swine, as well as the human in states of altered vascular function. The parasympathetic nervous system is believed to have a role in the pathophysiology of coronary vasospasm in patients with Prinzmetal's variant angina. There is also evidence supporting increased parasympathetic neural activity with endurance exercise training. The aim of these studies was to determine if endurance exercise training increases the coronary vascular responsiveness to intracoronary injections and infusions of acetylcholine in swine. Sensitivity to acetylcholine-mediated vasoconstriction was evaluated on the basis of the concentration required to achieve certain reductions in coronary blood flow in trained and sedentary pigs. Training was achieved by 10 weeks of treadmill running, after which all pigs were anesthetized with pentobarbital, and instrumented for an open-chest experiment. Intracoronary acetylcholine bolus injections (0.5 - 4.0 μg) were administered into the left anterior descending coronary artery at rest, and during simulated exercise, achieved by intravenous norepinephrine infusion, to determine acetylcholine's vasoconstrictive action. Acetylcholine (3.0 μg) was also

injected at one minute after cessation of exercise, and after muscarinic receptor blockade with intracoronary atropine injection (40 μg). Acetylcholine bolus injections caused similar percent reductions in coronary blood flow in both Exercise Trained and Sedentary groups at rest and during simulated exercise. However, Exercise Trained swine demonstrated a significantly ($p<0.05$) increased sensitivity to acetylcholine administered after cessation of exercise. A 3-fold difference in calculated acetylcholine concentration (19.5 ± 3.4 μM vs. 7.2 ± 0.5 μM) resulted in similar reductions in coronary blood flow (41.8 ± 7.6 % vs. 40.4 ± 9.3 %), in Sedentary and Exercise Trained swine, respectively. Atropine completely blocked acetylcholine-mediated vasoconstrictor responses. Acetylcholine was infused to achieve 10% and 30% reductions in coronary blood flow. Similar concentrations of intracoronary acetylcholine were required to achieve the 10% reduction (0.455 ± 0.092 μM vs. 0.397 ± 0.076 μM), but significantly ($p<0.05$) less acetylcholine (1.507 ± 0.270 μM vs. 0.648 ± 0.115 μM) was required to produce the 30% reduction in the Exercise Trained swine. These studies support the hypothesis that endurance exercise training enhances the sensitivity of the coronary vasculature to acetylcholine-mediated vasoconstriction in swine.

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by

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LIST OF ABBREVIATIONS

$(A\text{-}V)O_2$	Oxygen extraction
A	Arterial
ACh	Acetylcholine
CBF	Coronary blood flow
CVR	Coronary vascular resistance
dP/dt	Contractility
EPI	Epinephrine
HR	Heart rate
IC	Intracoronary
IV	Intravenous
kph	Kilometers per hour
L/P	Lactate/pyruvate
LV	Left ventricular
MABP	Mean arterial blood pressure
MVO_2	Myocardial oxygen consumption
NE	Norepinephrine
pCO_2	Partial pressure carbon dioxide
pH	- log hydrogen concentration
PIP	Peak intraventricular pressure
pO_2	Partial pressure oxygen

PRP	Pressure rate product
V	Venous

SIGNIFICANCE

The cardiovascular adaptations that occur with chronic endurance exercise have been studied extensively (Baldwin et al., 1977; Barnard, 1975; Blomqvist, 1983; Clausen, 1977; Cohen, 1983; Dowell, 1983; Frick, 1967; Hammond and Froelicher, 1985; Schaible and Scheuer, 1985; Scheuer, 1982; Scheuer and Tipton, 1977; Stone and Gwirtz, 1982; Thompson, 1982), but the modifications of coronary blood flow regulatory mechanisms remain uncertain. The mechanisms regulating coronary blood flow include physical, metabolic, and neural factors (Berne and Levy, 1981; Best and Taylor, 1985; Milnor, 1990). Physical factors include systolic ventricular contraction and perfusion pressure. Metabolic regulation involves the matching of oxygen supply with myocardial oxygen demand. As metabolism changes the levels of vasodilatory metabolites, which alter coronary vascular resistance and ultimately coronary blood flow, also change. Coronary blood flow is also neurally regulated by the sympathetic and parasympathetic nervous systems. The mechanisms of sympathetic innervation involve α-adrenergic-mediated coronary vascular smooth muscle contraction, and β-adrenergic-mediated relaxation. The net effect of these opposing actions is dependent on the dominant adrenergic receptor effect. The parasympathetic nervous system may cause muscarinic-mediated relaxation or contraction of vascular smooth muscle, depending on the animal species and presence of endothelial vascular disease. Examination of these regulatory mechanisms after physical conditioning may permit extrapolation to other altered coronary vascular states such as Prinzmetal's angina and atherosclerosis.

ACh, the parasympathetic nervous system neurotransmitter, is a potent vasoconstrictor of the coronary vasculature in the porcine model (Cowan and McKenzie, 1990; Ito et al., 1979; Nagata et al., 1985; Nakayama et al., 1988), as well as the human in states of altered vascular function (Ludmer et al., 1986; Miwa et al., 1988; Okumura et al., 1988; Vanhoutte and Cohen, 1984; Vita et al., 1990; Werns et al., 1989; Yasue et al., 1990). There is supporting evidence for an enhanced parasympathetic tone with exercise training as judged by increased atrial concentration and content of ACh in trained animals (De Schryver and Mertens-Strythagen, 1974; Herrlich et al., 1960; Smith and El-Hage, 1978), and bradycardia after immunologic sympathectomy (Tipton, 1965). The parasympathetic nervous system is also believed to play a role in the pathophysiology of coronary vasospasm (Yasue et al., 1974; Yasue et al., 1986). Common modifications of coronary blood flow regulatory mechanisms conceivably exist between endurance exercise training and certain vascular pathological states. Since exercise training is recommended for both the healthy individual and the cardiac patient to improve cardiovascular fitness, it is essential that the potential modifications of coronary blood flow regulatory mechanisms be elucidated.

PHYSICAL REGULATION OF CORONARY BLOOD FLOW

Physical factors regulating coronary blood flow include systolic ventricular compression and aortic blood pressure. During systole the caliber of the intramural vessels is decreased due to the force of the ventricular contraction. The extent to

which systolic compression affects coronary blood flow is determined by the strength of the contraction. During the cardiac cycle, there are phasic changes in coronary blood flow, with greatest flow occurring during diastole when the coronary vessels are not being compressed (Wiggers and Cotton, 1933; Kirk and Honig, 1964). The effect of systolic vascular compression on coronary blood flow is not uniform across the wall of the myocardium. Intramyocardial pressure is greatest in the endocardium, and less in the epicardium (Downey and Kirk, 1974). During diastole the inner wall receives a greater proportion of blood flow than the outer wall (Rubio and Berne, 1975). Therefore, both cardiac muscle layers receive equal flows over a cardiac cycle, regardless of the differences from systolic compression (Buckberg and Kattus, 1975).

Aortic blood pressure is responsible for providing the perfusion pressure of the myocardium. It is the driving force for coronary blood flow. Coronary blood flow remains relatively constant over a perfusion pressure range of 40 to 180 mm Hg due to the autoregulatory mechanisms that govern coronary circulation (Gregg and Fisher, 1963). Perfusion pressure changes elicit parallel changes in coronary vascular resistance in order to reestablish baseline coronary blood flow. According to Pouiselle's Law, flow is inversely proportional to the fourth power of resistance. Although perfusion pressure is an important determinant of coronary blood flow, the metabolic regulation of coronary resistance is the major determinant of coronary blood flow changes.

METABOLIC REGULATION OF CORONARY BLOOD FLOW

As the metabolic needs of the myocardium change, there is an inverse change in coronary vascular resistance, which is ultimately reflected as a change in coronary blood flow. If the work of the heart increases, its need for oxygen also increases. An increase in myocardial oxygen consumption can only occur if there is an increase in oxygen extraction and/or an increase in oxygen delivery. Since the myocardium's oxygen extraction is near maximal at rest, the increased oxygen demand is met by an increase in oxygen delivery (Ardehali and Ports, 1990).

An increase in the heart's metabolic rate results in the production of vasodilatory metabolites. These metabolites produce relaxation of the vascular smooth muscle and result in vasodilatation and an increase in coronary blood flow. When the work of the heart decreases, so does the production of metabolites and their dilatory effects on coronary vascular resistance, resulting in a reduction in coronary blood flow. The metabolic regulation of coronary vascular resistance is, therefore, the major determinant of coronary blood flow. The myocardial oxygen demands determine the magnitude of the blood flow supply.

Possible metabolic mediators include adenosine, molecular oxygen, hydrogen ion, carbon dioxide, potassium ion, lactate, histamine, increased osmolarity, and polypeptides (Ardehali and Ports, 1990; Feigl, 1983). Adenosine has received widespread support as being a primary mediator in the control of coronary blood flow in response to changing metabolic needs (Bardenheuer and Schrader, 1986; Berne, 1980; Feigl, 1983; Headrick and Willis, 1990; McKenzie et al., 1981;

McKenzie et al., 1983; Sparks and Bardenheuer, 1986). The adenosine hypothesis, proposed by Berne (1963), suggests that adenosine acts as the transmitter between the cardiac cell and the coronary vascular smooth muscle. As ATP is being utilized, it is broken down in the myocardial cell to adenosine. Adenosine accumulates as high-energy phosphate compounds are broken down, causes coronary vascular smooth muscle relaxation, and ultimately increases coronary blood flow.

During exercise, when cardiac performance is increased, there is increased oxygen demand requiring an increased oxygen supply. The necessary adjustment to increase coronary blood flow is primarily met by a decrease in coronary vascular resistance. McKenzie et al. (1981) demonstrated that this increase in coronary blood flow is mediated by adenosine. That adenosine plays a critical role in the local metabolic regulation of changes in coronary blood flow during hypoxia or ischemia is widely accepted (Berne, 1980; Feigl, 1983; Sparks and Bardenheuer, 1986). During physiological exercise the evidence for adenosine's role is not as strong (Bache et al., 1988), and other mediators possibly contribute to the resulting coronary vasodilatation. The heart consumes lactate as a source of fuel for ATP production. In addition to its role in myocardial energy production, it has vasodilatory properties as well. Lactate production is increased during conditions of increased myocardial work. Lactate accumulation results in vasodilation of the coronary vasculature to increase flow during such periods of increased myocardial oxygen demand.

NEURAL REGULATION OF CORONARY BLOOD FLOW

The neural control of coronary blood flow involves both the sympathetic and the parasympathetic branches of the autonomic nervous system. Although the major factors regulating coronary blood flow are local metabolic mechanisms, it has been demonstrated that there is a constrictor tone on the coronary vasculature, mediated by the sympathetic nervous system (Mohrman and Feigl, 1978). Resting tone of the coronary vascular smooth muscle does not appear to involve the parasympathetic nervous system (Cowan and McKenzie, 1990).

Sympathetic Nervous System

Sympathetic stimulation results in release of the neurotransmitter, norepinephrine. Norepinephrine activates both alpha- (α) and beta- (β) adrenergic receptors. Activation of the α-adrenergic receptor results in vascular smooth muscle contraction. The constrictor tone of the coronary circulation is mediated by α-adrenergic vasoconstriction. Stimulation of the sympathetic nerves activates both β_1- and β_2-adrenergic receptors. The β_1-adrenergic receptors are located throughout the myocardium, and when activated cause an increase in coronary blood flow, secondary to the primary response of increased contractility and heart rate. The increased inotropic and chronotropic state of the heart increases myocardial oxygen consumption, and therefore, increases coronary blood flow through metabolic mechanisms. The β_2-adrenergic receptors are located on the vascular smooth muscle of the coronary vessels, and when activated cause vascular smooth muscle relaxation

and an increase in coronary blood flow (Ginsburg, 1984). The β_2-adrenergic receptor responds to both norepinephrine and circulating, or non-neural, epinephrine with vascular smooth muscle relaxation. The affinity of the β_2-adrenergic receptor for epinephrine is greater than its affinity for norepinephrine (Milnor, 1990). Both the α-adrenergic and β_2-adrenergic receptors share the neurotransmitter norepinephrine, as well as coexist on the coronary vessel. The net effect of norepinephrine depends on the relative affinity and numbers of the two classes of receptors. In the coronary vessels, the predominate response to sympathetic stimulation of the heart is vasodilation (Feigl, 1983; Milnor, 1990). There is competition between β_2-mediated vasodilation, α-mediated vasoconstriction, and local metabolic influence, secondary to the β_1-mediated augmentation of myocardial oxygen consumption produced by increases in heart rate, contractility, and blood pressure.

Parasympathetic Nervous System

Stimulation of the parasympathetic nervous system causes release of the neurotransmitter, ACh. The corresponding receptor type is the cholinergic muscarinic receptor located on the coronary arterial vascular smooth muscle and endothelial cells. Prior studies (Feigl, 1969; Furchgott and Zawadzki, 1980; Tiedt and Religa, 1979) demonstrated endothelial mediated vasodilation to ACh, primarily in the canine model. Others have demonstrated species differences in the vascular smooth muscle's response to ACh *in vitro* (Kalsner, 1989). In the coronary arteries of sheep, pig, cattle, monkey, and man, ACh caused constriction (Ginsburg, 1984; Ito et al., 1979; Kalsner, 1985; Nakayama et al., 1988; Sakai, 1981). *In vivo* studies have

also demonstrated the vasoconstrictive actions of ACh and subsequent reductions in coronary blood flow (Cowan et al., 1987; Knight et al., 1986; McKenzie et al., 1988, Tondi et al., 1990). In several studies there was no difference in the vasoconstrictive response to ACh in the absence or presence of an intact endothelium (Ginsburg, 1984; Gräser et al., 1986) in porcine and human coronary arteries. However, other studies of coronary vessels have resulted in the current belief that ACh dilates normal human coronary vessels by releasing an endothelium-dependent relaxing factor, and constricts those vessels with some type of endothelial injury or dysfunction (Bossaller et al., 1987; Gordon et al., 1987; Ludmer et al., 1986; Okumura et al., 1987; Werns et al., 1989). Ludmer et al. (1986) concluded that a defective endothelial vasodilator function, due to the pathogenesis of atherosclerosis, and resulting in vasoconstriction, may be an important link to the mechanism of coronary vasospasm. Porcine coronary arteries respond to intracoronary ACh with constriction, regardless of endothelial disturbances (Gräser et al., 1986; Kalsner, 1985). The pig, therefore, is an excellent model for studying the vasoconstrictive actions of ACh.

Horio et al. (1986) observed nonuniform vasoconstrictive responses after intracoronary injection of ACh in human patients. Coronary arteriography revealed measurable diameter changes in adults with normal or almost normal arteriograms. The variability among responses could be indicative of varying degrees of atherosclerosis in these patients. Atherosclerosis produces endothelial injury and proliferation of smooth muscle cells, thereby altering the response to ACh. Yasue

et al. (1986) observed ACh induced, and atropine suppressed, attacks in patients with Prinzmetal's variant form of angina, implying the involvement of the parasympathetic nervous system. This type of angina is characterized as occurring spontaneously at rest, a time when parasympathetic nervous system activity is enhanced. The possibility exists that the parasympathetic nervous system plays a role in the pathogenesis of an attack of variant angina or coronary spasm (Yasue et al., 1974; Yasue et al., 1986). There appears to be a relationship between enhanced parasympathetic nervous activity and coronary vasospasm. If exercise training does augment this neural contribution to the regulation of coronary blood flow, then perhaps exercise training will augment the coronary response to ACh-mediated vasoconstriction. It also has been suggested that ACh has an inhibitory effect on the prejunctional release of norepinephrine (Cohen et al., 1984). This would limit sympathetic β_2-adrenergic-mediated relaxation of the coronary vascular smooth muscle, and result in a more pronounced vasoconstriction.

ENDURANCE EXERCISE TRAINING

Changes in the autonomic control of the heart that occur with chronic exercise include probable changes in both the parasympathetic and sympathetic nervous systems. Exercise bradycardia is a well known phenomenon when considering the trained heart. The basis for the decreased heart rate at both rest and submaximal workloads has been attributed to an enhanced parasympathetic component, an inhibition of the sympathetic component, and a combination of both changes of the

autonomic nervous system. The controversy continues as to which autonomic modifications are responsible for the bradycardia. Examination of the alterations in heart rate regulatory mechanisms with exercise training may reveal possible alterations in blood flow regulatory mechanisms as well.

Physical Regulation of Coronary Blood Flow

The physical regulators of coronary blood flow include wall tension and compression, and aortic blood pressure. Exercise training has uncertain effects on blood pressure. Most studies have noted small and insignificant changes. Other studies have demonstrated a decrease in diastolic blood pressure, and no significant change in systolic blood pressure (Boyer and Kasch, 1970). Choquette and Ferguson (1973) demonstrated decreases in both diastolic and systolic pressures. Martin et al. (1990) failed to show a decrease in blood pressure with exercise training in normotensive subjects, but exhibited an independent lowering of blood pressure in unmedicated hypertensive men. Other studies of hypertensive men have demonstrated a more significant lowering of blood pressure than their normotensive counterparts (Boyer and Kasch, 1970; Choquette and Ferguson, 1973). Astrand and Rodahl (1977) concluded that there is no consistent data to support exercise training induced reduction in perfusion pressure of the coronary vasculature in subjects without a pre-existing elevated blood pressure.

Endurance exercise training, such as treadmill exercise, causes a 50 percent increase in tension per unit of cross-sectional area and rate of force development (Tibbits et al., 1978) in the heart. The result is an increase in pump performance,

or an increase in myocardial contractile performance (Schaible and Scheuer, 1985). There is an enhanced inotropic state of the heart, i.e. increased contractility, reflected in an increase in dP/dt (Barnard, 1975; Clausen, 1977; Schaible and Scheuer, 1985). This is not always evident until the heart is stressed (Barnard, 1975). Clausen (1977) attributes the increase in contractility to increased activity of the enzymes coupled to the myocardial contractile proteins, induced by volume load to the heart, not pressure load. There is an increase in left ventricular end diastolic volume attributed to the exercise bradycardia, allowing more time for ventricular filling, and an increase in plasma volume (Nadel, 1985). The increase in ventricular filling, or increase in preload to the heart, results in increased stretch of the myocardium and more optimal crossbridge formation of the contractile machinery. According to Starling's law, the end result is a more forceful contraction, or an increase in contractility. This produces an increase in stroke volume (Barnard, 1975; Blomqvist, 1983; Dowell, 1983; Nadel, 1985) due to enhanced cardiac muscle contractile function.

Cardiac output is a function of heart rate and stroke volume: cardiac output = stroke volume X heart rate. Since endurance exercise training decreases resting heart rate and increases stroke volume, cardiac output is maintained without an increase in metabolic load to the heart. Resting coronary blood flow does not change appreciably with training due to little change in the metabolic demand of the heart. It has been noted, however, that there is a general increase in the coronary vascular tree with chronic exercise, manifested by an increase in the capillary to fiber ratio (Cohen, 1983; Scheuer, 1982). This results in greater coronary blood flow for

any given perfusion pressure.

Metabolic Regulation of Coronary Blood Flow

The trained state allows the subject to achieve greater levels of performance by the ability to consume greater amounts of oxygen (Schaible and Scheuer, 1985). In fact, the principal effect of training is to increase the maximal ability of the cardiovascular transport system to deliver oxygen to the tissues. Modifications of cardiovascular function with chronic endurance exercise may include alterations in the release of myocardial vasodilatory metabolites to parallel alterations in myocardial metabolism.

How closely the production and demand for ATP is matched is ultimately determined by substrate (oxygen) availability, waste (lactate) removal, and oxygen delivery (blood flow) (Idström. et al., 1986). During exercise the rate of oxygen demand may exceed the rate of oxygen supply in exercising skeletal muscle. The energy for continued work will then result from anaerobic metabolism, or glycolysis, and produce lactic acid, or lactate. Exercise training may increase the subject's tolerance to lactate as a result of anaerobic work (Robinson and Harmon, 1941). This increased tolerance may be due to an increased ability to accumulate lactate, or improved circulation to the exercising muscles which enables more rapid removal and distribution of lactate, and thus better buffering of this acid (Robinson and Harmon, 1941). Changes in sensitivity to acidosis may also occur with repetitive bouts of exercise that occur with training (Robinson and Harmon, 1941). That chronic exercise enables the subject to exercise with a decreasing proportion of

energy coming from anaerobic mechanisms, implies that the maximal ability to consume oxygen requires improvements in circulation and oxidative processes in the exercising muscles. The subsequent decline in blood lactate during submaximal workloads with training has been attributed to increased blood clearance (Donovan and Brooks, 1983) and/or decreased production (Holloszy and Booth, 1976; Hurley et al., 1984). Donovan and Brooks (1983) concluded that trained animals are more capable of matching their rates of lactate removal to lactate production than untrained animals. Untrained animals have to tolerate an elevated blood lactate to sustain a given removal rate. Decreased production of lactate could be a result of a greater proportion of pyruvate being channeled into the mitochondrial oxidative pathway and less into lactate (Hurley et al., 1984).

Changes in lactate metabolism in skeletal muscle with exercise training may also be reflected in cardiac muscle. After exercise training there is a lower lactate concentration at the same workload as before training, possibly indicating that the heart is a better oxygen supplier and a better lactate remover (Karlsson et al., 1972). The heart consumes lactate as a source of fuel for ATP production. Karlsson et al. (1972) reported that there is a faster metabolism of lactate with physical training in exercising skeletal muscle. Perhaps the heart utilizes lactate more proficiently as well.

During a myocardial ischemic event, such as reduced coronary blood flow, lactate is expected to accumulate, since lactate is an indicator of oxygen deficiency. With endurance exercise training, lactate is expected to be consumed at a faster rate

by the myocardium and not accumulate, providing the myocardium with more effective oxygen transport and diminished anaerobic energy yield. Lactate's role as a coronary vasodilator may be enhanced as well, to increase coronary blood flow and limit the severity of myocardial ischemia.

Endurance exercise training results in the development of an improved supply-demand balance in the heart and a more efficient myocardial performance. Whether this adaptation to exercise is due to alterations in the metabolic regulation of coronary blood flow is still uncertain.

Neural Regulation of Coronary Blood Flow

Sympathetic Nervous System

During strenuous exercise, circulating levels of catecholamines increase and myocardial blood flow is appreciably increased, indicating an apparently greater influence of β_2-adrenergic receptors. Exposure of cells to high concentrations of an active hormone results in a subsequently decreased sensitivity termed desensitization, refractoriness, tolerance or tachyphylaxis (Lefkowitz et al., 1984). Desensitization can be accompanied by down-regulation, a decrease in the number of receptors, destruction or internalization of receptors. Desensitization can also be attributed to uncoupling of the receptor from the cellular response, or a diminished effect due to repeated or prolonged exposure to the ligand. It has been shown that a single bout of dynamic exercise is sufficient to produce a significantly decreased chronotropic responsiveness to isoproterenol, a β-agonist (Friedman et al., 1987). This was an

acute and transient desensitization of cardiac β-adrenergic receptors. Butler et al. (1983) described the desensitization process as having a protective role in limiting the initial cardiac accelerative response that occurs with exercise. Long-term treadmill running was shown by Hammond et al. (1988) to result in substantial down-regulation of atrial β-adrenergic receptors, and an enhanced responsiveness of these receptors to catecholamines. This study showed that in response to simulated exercise with intravenous administration of isoproterenol, a β-agonist, there was an initial increase in β-adrenergic receptor density and responsiveness. This was followed by a significant decrease in both these factors below pre-exercise values. Responsiveness was evaluated on the basis of cyclic-AMP production in response to an increase in Gs (stimulatory guanine nucleotide-binding protein) concentration. An increase in Gs in response to isoproterenol administration suggests altered signal transduction, which may contribute to altered adrenergic responsiveness with chronic endurance exercise (Hammond et al. 1988). Cousineau et al. (1977) demonstrated that physical training results in diminished sympathetic responses for a given level of exercise. Lower arterial catecholamine increases at various workloads were associated with diminished heart rate and systolic blood pressure responses.

Down-regulation of β-adrenergic receptors has gained support as an important mechanism underlying diminished chronotropic responsiveness with chronic exercise (Butler et al., 1982; Hammond et al., 1987; Savin et al., 1983). Hammond et al. (1987) revealed a decrease in β-adrenergic receptor number in the right atrium following chronic exercise, with no changes in left ventricular receptors. The

importance of right atrial membranes is due to their proximity to the sinus node and, therefore, are most closely associated with the chronotropic response. In addition, there was no noted difference in muscarinic cholinergic receptor number in right atrial membranes. Both Hammond et al. (1987) and Butler et al. (1982) noted that these changes in adrenergic receptor density were proportional to changes in physical fitness. Further support for alterations in the sympathetic component being responsible for exercise bradycardia is found in studies of sympathectomized animals (Sigvardsson et al., 1977), where no training bradycardia was exhibited. Bove (1989) suggests that training causes an increase in conjugated dopamine in the blood. This catecholamine serves as a storage pool for rapid conversion to norepinephrine during exercise.

Not all investigators agree that the β-adrenergic receptor has a role in inducing training bradycardia. Nylander (1985) demonstrated that cardioselective and non-selective β-blockade during treadmill training had no effect on the development of training bradycardia. β-Blockade was also shown not to impair the training-induced increase in the maximum O_2 uptake (Svedenhag et al., 1984). Sigvardsson et al. (1977) demonstrated that β-adrenergic receptor sensitivity was not altered by physical training. Others claimed that since β-blockade still exhibited a training bradycardia, although less than those without blockade, an increase of the parasympathetic tone is a more likely explanation for the observed exercise-induced bradycardia (Vanhees et al., 1982). Raab et al. (1960) noted a decrease in the cardiac sympathetic tone as measured by the chronotropic state of the heart, and

length of the isometric contraction period. These decreases in heart rate and shortening of ventricular tension period, were in proportion to the degree of habitual exercise; thus lending support to the cholinergic preponderance of the trained athlete's heart.

Parasympathetic Nervous System

Research supporting the enhancement of the parasympathetic nervous system with chronic exercise includes studies indicating an increase in the cholinergic neurotransmitter, ACh, to reflect an increase in parasympathetic neural activity with training. An increase in ACh concentration (De Schryver and Mertens-Strythagen, 1974; Herrlich et al., 1960) and content (De Schryver and Mertens-Strythagen, 1974) has been demonstrated in atrial extracts. The question can be raised, does an increase in ACh in the heart only reflect an increase in the parasympathetic activity on the heart? The increase in vagal neurotransmitter concentration could be due to either an increased synthesis (Ekström, 1974), decreased enzymatic degradation by acetylcholinesterase, and/or an increase in vagal efferent activity (Åstrand and Rodahl, 1977; Robinson et al., 1966). Ekström (1974) demonstrated an increase in the activity of the enzyme choline acetyltransferase in the atria of exercise trained rats, indicating an increase in the synthesis of ACh. Smith and El-Hage (1978) demonstrated bradycardia in isolated atria from exercise trained rats, which after atropine administration, resulted in a significant increase in heart rate. This study lends support to the contribution of non-neural ACh in producing exercise

bradycardia. Additional support that bradycardia is related to non-neural factors was demonstrated by Tipton and Taylor (1965). In this study, the exercise trained animals exhibited a lower heart rate than their sedentary counterparts, and in response to intraperitoneal atropine, the untrained animals demonstrated a significantly greater cardiac acceleration. Animals with more available ACh (from both neural and non-neural sources) were expected to demonstrate less of a cardiac response to atropine. Non-neural ACh serves to supplement the effect of the ACh from the vagal endings. In contrast, Maciel et al. (1985) did not show significant changes in heart rate with training after parasympathetic blockade with atropine. Tipton (1965) produced bradycardia in vagotomized and immunologically sympathetomized rats, supporting the involvement of non-neural ACh within the myocardium in exercise bradycardia. Barnard (1975) concludes that the long-term effects of exercise on cardiac function support the primary involvement of the parasympathetic nervous system in training bradycardia.

Claims also exist that while the resting bradycardia is due primarily to enhanced parasympathetic tone, the bradycardia at submaximal workloads is due to reduced sympathetic tone (Ekblom et al., 1973; Frick et al., 1967). Lower levels of circulating catecholamines during submaximal workloads after training lend support to this conclusion (Hartley, 1975). Bogenhagen et al. (1990) conversely demonstrated that the parasympathetic nervous system significantly restrains heart rate during an acute bout of moderately heavy submaximal exercise.

Exercise bradycardia may be due to not only changes in autonomic nervous

activity, but changes in pacemaker sensitivity to postganglionic neurotransmission and/or a lowering of the spontaneous intrinsic rhythm of the sinoatrial node. According to Bolter et al. (1986), training induced a modification in the intrinsic sinoatrial nodal rate, without influencing norepinephrine induced chronotropic responses. This intrinsic change is refuted by Williams et al. (1981), who demonstrated decreased sensitivity of the sinus node to β-adrenergic stimulation. The possibilities exist that there is a change in the balance of cardiac autonomic nervous activity, an alteration in intrinsic rhythm of the sinoatrial node, and an alteration in the response of the sinoatrial node to autonomic neurotransmitters, ACh and norepinephrine. There is no question that exercise bradycardia exists, whether it is mediated directly at the sinus node, or through alterations in the central output to the sinus node.

Coronary Blood Flow Studies

Coronary blood flow studies have demonstrated that chronic exercise decreases the α-adrenergic receptor mediated vasoconstrictor tone of the coronary vasculature, resulting in an apparently greater role for the coronary vascular β_2-adrenergic receptor in controlling vascular tone (DiCarlo et al., 1988; Gwirtz and Stone, 1984). These studies support exercise training induced alterations in the neural control of the coronary circulation. Liang and Stone (1983) demonstrated a change in the neurogenic control of the coronary vasculature by a reduction in sympathetic neural activity on the coronary resistance vessels with training during submaximal exercise. Oltman et al. (1990) reported an altered response of the

coronary vessels to sympathoamines with exercise training, depicted by a decreased sensitivity to norepinephrine.

Most likely, the mechanism responsible for bradycardia following chronic endurance exercise involves parasympathetic activity, together with an attenuation of sympathetic nervous activity. In other words, there appears to be an alteration in the vascular tone of the coronary blood vessels manifested in a decreased α tone, which may result in greater β_2-mediated vasodilation with sympathetic stimulation. Due to increased ACh, neural and/or non-neural, a change in muscarinic associated vascular responsiveness may also occur. Altered β_1-adrenergic responsiveness (Butler et al., 1982; Friedman et al., 1987; Hammond et al., 1987; Hammond et al., 1988; Savin et al., 1983), is manifested in receptor down-regulation and reduced chronotropic responsiveness to β-adrenergic agonists. Increased sensitivity to norepinephrine may result in greater β_2-mediated vasodilation. Alteration of coronary vascular tone with exercise training might also affect the coronary vasculature susceptibility to vasospasm. Patients with Prinzmetal's variant angina are believed to have increased susceptibility to vasospasm when the parasympathetic nervous system is enhanced (Yasue et al., 1979). With an enhanced parasympathetic component due to exercise training, the vasoconstrictive response to ACh in the porcine model is expected to be magnified.

CLINICAL RELEVANCE

Robinson et al. (1966) characterized the control of heart rate by the autonomic nervous system in man. They proposed that at rest, the parasympathetic component is the dominant influence on heart rate. In response to mild exercise, however, heart rate increases due to withdrawal of resting parasympathetic inhibition. With higher levels of exercise, heart rate increases in response to heightened sympathetic drive. During recovery from exercise, there is a phase of "vagal recapture," where sympathetic activity decreases gradually, and parasympathetic activity increases (Savin et al., 1983) to reestablish resting conditions. Friedman and Friedman (1990) demonstrated in healthy young men that if exercise was abruptly terminated, not only did heart rate decrease as expected, but asystoles occurred suddenly during this "vagal recapture" period. This was rarely seen if exercise was terminated gradually. Arai et al. (1989) demonstrated a marked reduction of autonomic modulation of heart rate in patients with heart failure (deranged cardiac autonomic function and reduced heart rate variability) and after cardiac transplant (no direct autonomic connections to the heart and nearly absent beat-to-beat heart rate variability) to support the progressive withdrawal of vagal activity during exercise, with a gradual increase during recovery in normal subjects. Sadaniantz et al. (1988) linked the reestablishment of parasympathetic dominance during recovery from exercise to involvement in post-exercise coronary artery spasm. In patients with minimal coronary artery disease, vasospasm occurred soon after exercise termination, as measured by ST-segment elevation, angina, narrowing of coronary arteries as

revealed by coronary angiography, and mild hypokinesis. If exercise training does enhance parasympathetic nervous system activity, then there may be an increased likelihood that trained individuals would be more susceptible to coronary artery spasm, especially with abrupt cessation of exercise.

Prinzmetal's variant angina is characterized by chest pain at rest, associated with transient ST-segment elevation in the electrocardiogram, and believed to be caused by coronary arterial spasm (Prinzmetal et al., 1959; Prinzmetal et al., 1960). Since parasympathetic nervous system activity is enhanced at rest, it may be related to the pathogenesis of variant angina or coronary spasm (Araki et al., 1983; Becker et al., 1987; Yasue et al., 1986). Yasue et al. (1986) tested the induction of coronary spasm in patients with variant angina with intracoronary injections of ACh and found spasm, chest pain, and ST-segment elevation or depression (indicators of myocardial ischemia). The attack was suppressed by atropine, confirming the involvement of the muscarinic receptor. Weiner et al. (1978) reported a new manifestation of variant angina - ST-segment elevation and chest pain occurring only during recovery after an exercise test. This response may be related to the alterations in the autonomic balance during recovery after exercise as noted previously. This supports the increased susceptibility of coronary spasm when the parasympathetic nervous system's activity is predominant.

Changes in autonomic nervous activity that occur with chronic exercise have also been attributed with providing electrical stability to the heart (Billman et al., 1984). Cardiac electrical instability, reflected in life-threatening arrhythmias such as

ventricular fibrillation, is a primary factor responsible for sudden death. Myocardial stability is enhanced by reducing sympathetic efferent activity and increasing vagal efferent activity. This property was demonstrated by Schwartz et al. (1984) in dogs with healed myocardial infarction, that underwent acute myocardial ischemia, which elicits high sympathetic activity. Those animals with strong vagal reflexes had a reduced incidence of ventricular fibrillation during the acute myocardial ischemia incident. If exercise training does enhance cardiac parasympathetic activity, then it can provide a means for preventing ventricular fibrillation leading to sudden death. Waxman et al. (1988) described a healthy man with an idioventricular rhythm that could consistently be depressed by increased vagal tone, or acceleration by increased sympathetic tone. Suppression of the pacemaker's automaticity by enhanced vagal tone provided some stability to the pacemaker's predisposition to ventricular tachycardia.

The controversy over prescribing regular physical activity for patients after a myocardial infarction is continual. The National Exercise and Heart Disease Project attempted to identify the benefits from exercise on mortality and cardiovascular morbidity in such patients (Shaw, 1981). Exercise was shown to reduce the incidence of death by 37 percent. Although there was no apparent benefit from exercise in relation to morbidity, no harm was evident either. The implications of the study were limited to the acknowledgement that a trial on the worth of exercise for cardiac patients is feasible, and that the benefits of an exercise program in the patients that were studied remains inconclusive, mainly due to the size limitations of the study.

Rechnitzer et al. (1983) studied the effects of high intensity exercise for prevention of recurrent myocardial infarction in man. The results of the Ontario Exercise-Heart Collaborative Study indicated that there was no reduction in the risk of myocardial infarction in patients that participated in a high intensity exercise program vs one designed to produce a minimal training effect. Both of these studies have failed to report significant protection from recurrent myocardial infarction incidence with exercise training, in spite of some favorable trends.

The potential benefits and/or detriments of chronic endurance exercise, such as running or swimming, remain controversial. Alterations in autonomic control of the heart and its vasculature are of major concern when attempting to evaluate clinical events such as post-exercise ischemia, sudden death, and recurrence rate of myocardial infarction after physical conditioning. Cantwell (1985) describes electrocardiograph abnormalities in endurance athletes such as transient sinus pauses, atrial-ventricular dissociation, and Wenckebach atrioventricular block reflecting an increase in vagal tone. Support exists for either an enhanced parasympathetic tone and/or inhibited sympathetic tone with exercise training. In order to determine alterations of the coronary vascular response under exercise trained conditions, it is essential to examine potential modifications of neural and metabolic regulatory mechanisms of coronary blood flow.

RATIONALE

Chronic endurance exercise is known to cause alterations in cardiovascular function, including bradycardia at rest and during submaximal exercise. The interaction between sympathetic and parasympathetic nerve involvement in cardiovascular function with chronic endurance exercise training is the subject of intense investigation. There is increasing support for enhancement of the parasympathetic nervous system as the major adaptation responsible for bradycardic alterations in heart rate with physical training. However, there is conflicting evidence supporting the inhibition of the sympathetic branch of the autonomic nervous system as well. These neural changes which alter the chronotropic state of the trained heart, also affect the regulation of the coronary vasculature. There is a decreased α-adrenergic vasoconstrictor tone resulting in more pronounced β_2-adrenergic receptor mediated vasodilation. With an enhanced parasympathetic component, there is also an expected change in muscarinic associated vascular responsiveness.

The neurotransmitter for the parasympathetic branch of the autonomic nervous system is ACh. ACh is a potent vasoconstrictor of the coronary vasculature in both swine and human models. It acts through muscarinic receptors located on the coronary vascular smooth muscle to cause an increase in coronary vascular resistance, and a reduction in coronary blood flow. The purpose of these studies was to determine whether alterations in the parasympathetic regulation of coronary blood flow, due to endurance exercise training, would result in enhanced ACh-mediated vasoconstriction in the porcine model.

To establish a state of cardiovascular fitness, swine were exercised on a treadmill for a period of ten weeks at an intensity of seventy five percent of maximal heart rate. The coronary responsiveness of endurance exercise trained pigs to ACh was compared to sedentary controls. Intracoronary bolus injections of ACh were administered into the left anterior descending coronary artery at rest, during simulated exercise achieved by intravenous infusion of norepinephrine, and at one minute after cessation of exercise. In addition to bolus injections, two intracoronary infusions of ACh were administered to achieve mild (ten percent) and moderate (thirty percent) reductions in coronary blood flow. Sensitivity to ACh mediated vasoconstriction was evaluated on the basis of the concentration required to achieve a particular reduction in blood flow.

Plasma lactate, pyruvate, and catecholamines were measured to determine potential alterations in metabolic regulators of coronary blood flow. Blood gases were measured to determine alterations in myocardial oxygen consumption. Coronary blood levels of total cholinesterase were determined to evaluate if the effects of exogenously administered ACh are enhanced with exercise training due to decreased enzymatic degradation.

The parasympathetic autonomic nervous system is believed to be enhanced in Prinzmetal's variant angina, and therefore, a possible candidate for mediator of coronary vasospasm. If exercise training accentuates the activity of the parasympathetic nervous system, then the trained heart may be more susceptible to coronary vasospasm at times when the parasympathetic nervous system is

predominant. An especially vulnerable time would be at cessation of exercise when parasympathetic activation is replacing the predominate exercise-induced sympathetic activation. Post-exercise vasoconstriction and constriction at rest are the major concerns due to the domination of the parasympathetic nervous system in regulating cardiac function at these times.

The results of these studies support the hypothesis that endurance exercise training enhances the sensitivity of the coronary vasculature to ACh-mediated vasoconstriction in the porcine model.

MATERIALS AND METHODS

EXPERIMENTAL DESIGN & TECHNIQUES

It is essential to choose an animal model from which appropriate physiological comparisons to humans can be made. In need of consideration for these studies are similarities in cardiac function and structure, and response to exercise. It has been documented that the pig is an excellent model for both coronary and exercise physiology studies (Bloor et al., 1986; Hughes, 1986; Laughlin et al., 1989; McKirnan et al., 1986; Sanders et al., 1977; White et al., 1986) . Justifications for the use of swine in this study included the following:

- heart size and relevant weight (heart size to body weight ratio) are similar to humans
- coronary artery anatomy is similar to the human heart
- coronary collateral flow is sparse, like the human
- responds to exercise stress in a similar manner to humans
- adaptability to treadmill exercise is excellent
- available at relatively low cost.

Immature male Yorkshire swine approximately 3-4 months old, and weighing 15-20 kg were used as the animal model for this study.

After a 1 week stabilization and adaptation period, all pigs were subjected to a treadmill stress test in order to determine physical condition and group homogeneity. The pigs had never been exposed to the treadmill before the first

treadmill stress test. Heart rate was monitored during the following stages of the test (kph = kilometers per hour):

1) Rest

2) 5 kph at 0% grade - 5 min

3) 5 kph at 10% grade - 5 min

4) 7 kph at 10% grade - 3 min (which was sufficient time to achieve maximum heart rate)

5) Recovery - 5 min

Immediately after the treadmill was stopped, a venous blood sample was obtained from a marginal ear vein for plasma lactate determination. After this test the animals were randomly assigned to an exercise or sedentary group. The objective of the training protocol was to exercise on a motorized treadmill at a workload intensity of 75% of maximum heart rate for 10 weeks. Treadmill grade was kept constant at 0% grade during training, while the treadmill speed was varied to achieve the appropriate workload. The maximum heart rate was determined during the treadmill stress test. Periodic ECG testing during the 10 weeks training period was done to ensure that the desired training intensity was being achieved. The training protocol was modeled after McKirnan et al. (1986) with the specifics as follows:

- pigs ran 3 days per week for 10 weeks,

- exercise duration starting at 20 min/day, increasing to 30 min/day by the end of week 2,

- duration of running was increased 10 min/week until reaching a maximum duration of 60 min by end of week 6,

- 60 min/day duration continued until the end of week 10.

The sedentary group had no further exposure to treadmill running. The sedentary pigs remained in the animal facility for the 10 week period. At the end of 10 weeks both sedentary and exercise trained animals were subjected again to a treadmill stress test to determine changes in heart rate at rest and during submaximal exercise, as well as changes in venous plasma lactate values. Lactate clearance, production and turnover are altered with exercise training (Donovan and Brooks, 1983; Hurley et al., 1984; Karlsson et al., 1972). McKirnan et al. (1986) demonstrated that mixed venous lactate was lower at maximal exercise after training in moderately and intensely exercised Yucatan swine. The moderately exercised pigs had serum lactate values of 19.8 ±2.8 mM (pre-training) and 12.9 ±3.0 mM (post-training), in comparison to this study where the serum lactate values in the exercise pigs were 14.0 ±2.8 mM (pre-training) and 10.3 ±2.7 mM (post-training). Venous lactate sampling has provided an indication of successful training in previous studies (McKirnan et al., 1986, Robinson and Harmon, 1941), and was used as an indicator in this study as well. Two days later the animals were anesthetized for an acute experiment.

The animals were sedated with an intramuscular injection of ketamine (12 mg/kg), and a 20 gauge angiocath introduced into a marginal ear vein for administration of pentobarbital (0.5 g/ml, as required) to establish anesthesia. The trachea was intubated with an 8 or 9 mm endotracheal tube. Electrodes were placed in both right and left legs for ECG recording. The left femoral artery and vein were catheterized for arterial blood samples and intravenous route for

administration of agents (pentobarbital, nitroglycerine, norepinephrine), respectively. Under fluoroscopy, cardiac catheters were placed in the left ventricle, great cardiac vein, and left anterior descending coronary artery (LAD). Renografin-76 (Squibb Diagnostics) was used to verify placement of all cardiac catheters. An 8F Millar Mikro-tip dual pressure transducer catheter was advanced from the right femoral artery to the left ventricle. The catheter was positioned so that the distal pressure transducer was in the ventricular cavity to record intraventricular pressure and dP/dt, and the proximal pressure transducer was in the root of the aorta to record coronary perfusion pressure. A 7.5F Sones catheter was advanced from the left jugular vein to the great cardiac vein for coronary venous blood samples. A 9F sheath (Cordis) was placed into the left carotid artery, and an 8F Multipurpose B-1 Marathon Guiding Catheter (Baxter) was advanced through the sheath to the main left coronary artery for advancement of an infusion catheter into the proximal LAD. A USCI 0.014 inch floppy tipped guide wire was advanced through the guiding catheter into the proximal LAD, and a 2.5F infusion catheter advanced over the wire to a point in the proximal LAD that was free of branches. This catheter provided access to a coronary vessel for intracoronary injections and infusions. After placement, the LAD catheter was filled with heparin (1000 units/ml, 3 ml total volume), and 0.1 ml heparin was injected every 30 min until the experiment began in order to maintain catheter patency and prevent clot formation. An intravenous infusion of nitroglycerin (0.25 mg/ml, as required), a vasodilator, was administered during fluoroscopy to obtain a 5-10 mmHg reduction in systolic blood pressure (50 μg/min). The purpose

of this infusion was to prevent coronary vessel spasm during cardiac catheter placement. The onset of action of intravenous nitroglycerin is rapid (minutes), but its hemodynamic effects are quickly reversed with discontinuaiton of its infusion (Katzung and Chatterjee, 1987). It took approximately 2 hours from the time the animal was anesthetized, until completion of catheter and electrode placement.

Arterial and coronary venous blood samples were taken before the thoracotomy procedure for measurement of total cholinesterase and catecholamines. The chest was then opened with a left lateral thoracotomy at the fourth intercostal space, and the animal ventilated with a Harvard respirator at 8-10 breaths/minute, 450 ml tidal volume. Atelectasis was prevented with a 3-5 cm H_2O positive end expiratory pressure. The pericardium was incised longitudinally for access to the epicardial vessels. The LAD was isolated and a circumferential electromagnetic flow probe placed around the LAD to record phasic and mean coronary blood flow. Coronary blood flow measurements were determined utilizing an electromagnetic flowmeter (Carolina Medical Electronics, Model FM501). The flowmeter operates on the principle of electromagnetic induction, providing blood flow information with 10% accuracy, without impeding coronary blood flow. The flow probe contains an electromagnet which produces a magnetic field across the coronary vessel. Motion of the blood through the magnetic field generates an induced voltage proportional to velocity. For a given vessel diameter, the induced voltage is also proportional to the flow rate. If anatomically possible, a second flow probe was placed around the circumflex coronary artery to serve as an internal control (5/11 Sedentary animals,

2/11 Exercise Trained animals). A 30 minute stabilization period followed completion of the instrumentation, during which time blood gases and pH were measured and, if necessary, corrected to within normal physiological limits (pO_2, 80-90 mmHg; pCO_2, 30-40 mmHg; pH, 7.4-7.5). This was accomplished by adjusting the tidal volume and respiratory rate of the respirator apparatus, or oxygen supplementation. Hemodynamic parameters including heart rate, arterial blood pressure, intraventricular pressure, dP/dt, and phasic and mean LAD coronary blood flow were continuously monitored. Arterial and coronary venous blood were collected at various stages to measure the following myocardial parameters: lactate, pyruvate, pO_2, pCO_2, O_2 content, and hematocrit.

After the stabilization period, control blood samples were collected for measurement of myocardial parameters. This was followed by random intracoronary (IC) bolus injections of ACh in the dose range of 0.5 - 4.0 μg (in 0.1 - 0.5 ml volume) to determine the responsiveness to ACh at rest. IC bolus injections of saline (vehicle control for ACh) were made to test the volume effects of the ACh injections (0.5 ml).

After completion of the resting ACh bolus injections, collection of blood samples were repeated to obtain pre-exercise values. To simulate a moderate exercise workload, an intravenous (IV) infusion of norepinephrine (0.1 mg/ml) was given until a 60-70% increase in pressure-rate product (PRP = peak intraventricular pressure x heart rate) was obtained. PRP is a mechanical correlate of cardiac work, an indicator of O_2 demand, and thus O_2 consumption. Once this level of work had stabilized (approximately 5-8 minutes), blood samples were taken. Random IC bolus

injections of ACh in the dose range of 0.5 - 4.0 ug were given during the increased workload to determine the coronary vascular responsiveness to ACh during simulated exercise. One minute after completion of the norepinephrine infusion, a 3.0 μg IC bolus injection of ACh was given to assess post-exercise reductions in coronary blood flow. The animal was then given a 30 minute rest period for hemodynamic parameters to return to baseline.

The next phase of the experiment included intracoronary infusions of saline and ACh. Saline infusion served as vehicle control for the ACh infusions, and determined the volume effect of an IC infusion of average rate (1.75 ml/min). An IC infusion of ACh was then administered (rate of infusion approximately 1.0 ml/min), until a 10% reduction in LAD coronary blood flow was achieved. When the reduction had stabilized, blood samples were taken. The infusion was stopped and the animal given time to return to original hemodynamic status. A second IC infusion of ACh was given (rate of infusion approximately 1.5 ml/min) until a 30% reduction in LAD coronary blood flow had been achieved, and blood samples taken when there was stable flow at the reduced rate. After completion of sample collection the infusion was stopped and time allowed for hemodynamic parameters to reach baseline values. The ACh infusions took approximately 3-5 minutes to reach a stabilized reduced blood flow. Approximately fifteen minutes was allowed between ACh infusions for hemodynamic parameters to return to baseline.

To verify that acetycholine's actions were via the muscarinic receptor, an IC bolus injection of atropine (40 μg) was administered, and the response to a 3.0 μg

dose of ACh was measured. In an atropine dose response study done in our laboratory, 5×10^{-4} M (40 μg) atropine blocked the vascular effects of doses up to 20 μg ACh. Euthanasia was then performed with an overdose of IV pentobarbital (0.5 gram in 10 ml volume).

The LAD was infused with India ink as the animal was being euthanized in order to dye the region of LAD perfusion. After the animal was euthanized, the heart was removed and the black stained perfused region cut out of the heart and weighed in order to normalize coronary blood flow per 100 gram tissue weight. In addition, the weights of the heart and left ventricle (LV) were measured for calculation of heart/body weight and LV/body weight ratios for determination of cardiac hypertrophy.

CHEMICAL ANALYSIS

Blood gas status including pO_2, pCO_2, pH, and O_2 content was determined with a Radiometer ABL-3 or Instrumentation Laboratories 1306 Blood Gas Analyzer and Radiometer OSM3 Hemoximeter. Blood gas samples were collected anaerobically in chilled, heparinized syringes and analyzed promptly. Hematocrit determination followed blood gas analysis. Blood was taken up into capillary tubes and centrifuged for 5 min with an IEC MB Micro Hematocrit Centrifuge. Hematocrit values were read utilizing an IEC Micro Capillary Reader.

Plasma catecholamines were analyzed using HPLC with electrochemical detection as described by Bioanalytical Systems, Inc. Since the catecholamines, norepinephrine (NE) and epinephrine (EPI), are both heat and light sensitive, the arterial and venous blood samples were kept on ice and in the dark. Blood samples (4.0 ml) were collected into ice chilled tubes containing preservative (80 μl of glutathione solution - 60 mg glutathione in 1.0 ml 0.24 M EGTA), centrifuged at 10,000 rpm for 10 minutes at 4° C. The plasma was separated and stored frozen at -20° C until analysis. At assay time 1.0 ml plasma, 2000 pg 3,4-dihydroxybenzylamine hydrobromide (DHBA), 50 mg acid washed alumina (AAO), and 0.5 ml Tris buffer (1.5 M Tris, 0.06 M EDTA, pH 8.6) were vortexed and shaken for 5 min. The adsorption of the catecholamines to the AAO is pH dependent at pH 8.5. The pH was adjusted by the volume of tris buffer added. DHBA served as the internal standard. After shaking and allowing the alumina to settle, the supernatant was aspirated. The alumina was washed with 1.0 ml distilled H_2O (pH 7.0), shaken for

5 min, and aspirated 4 additional times. Following this washing procedure, 1.0 ml distilled H_2O was added to the alumina, and the alumina slurry transferred to a centrifugal microfilter loaded with a 9 mm RC58 membrane (RC = regenerated cellulose), 0.2 micron pore size, and centrifuged at 1000 x g for 1 min. The receiver tube on the filter apparatus was replaced and 200 μl 0.1 M perchloric acid (PCA) added to the sample compartment. The filter apparatus was vortexed briefly, allowed to stand for 5 min, vortexed briefly again, and centrifuged at 1000 x g for 1 min. The mixing is important for maximal desorption of the catecholamines from the AAO. The desorption occurs at pH 1.0. The acidic extract in the receiver tube contained the catecholamines and was ready for HPLC injection. A standard sample consisting of 1.0 ml phosphate buffer, 1500 pg NE, 1500 pg EPI, and 2000 pg DHBA was also assayed. To calculate the catecholamine concentrations, peak height ratios (relative to the internal standard DHBA) for unknown plasma samples were compared to those for the standard sample whose original concentrations are known. The equation for calculating NE was:

$$(\text{concentration NE})_{UNK} = \frac{(\text{NE/DHBA})_{UNK} \quad X \quad (\text{concentration NE})_{KNOWN}}{(\text{NE/DHBA})_{KNOWN}}$$

where $(\text{concentration NE})_{KNOWN} = 1500$ pg.

Epinephrine was calculated in a similar fashion.

A blank sample of 1.0 ml phosphate buffer with no plasma or standards was assayed as described above to assure that reagents did not contain extractable contaminants which may produce interference peak(s) on the chromatogram. The

HPLC method utilized electrochemical detection (BAS LC-4B Amperometric Detector) of the analytes separated by a Phase II ODS 3 μm reverse phase column, 100 x 3.2 mm. Mobile phase (0.075 M NaH_2PO_4, 1.0 mM EDTA, 0.9 mM sodium octyl sulfate, 1.5% acetonitrile, pH 3.0 with H_3PO_4) was pumped through the column at 1.0 ml/min via Waters Model 501 HPLC pump, and the sample injected using a Waters 712 Wisp injector. The analyte was detected as it passed the glassy carbon electrode surface, and its chemical form changed by the mechanism of oxidation to o-quinone. This resulted in a current that developed at a specific applied potential (+0.65 volts vs. Ag/AgCl) as it passed over the glassy carbon electrode.

$$\text{Catecholamine} \rightarrow \text{o-quinone} + 2H^+ + 2e^-$$

The limit of sensitivity of this method for NE is 25 pg, and 50 pg for EPI.

Cholinesterase activity was analyzed by colorimetric determination according to Ellman et al. (1961). This is a nonspecific assay which measures total esterase activity (red cell cholinesterase or acetylcholinesterase, and plasma cholinesterase or pseudocholinesterase). Arterial and venous blood samples were taken before the thoracotomy surgical procedure. Ten μl of blood were collected into a chilled tube containing 6.0 ml 0.1 M pH 8.0 phosphate buffer and refrigerated until assay time. The spectrophotometric measurement (Beckman DU-65 Spectrophotometer) is based on measurement of the rate of production of thiocholine as acetylthiocholine is hydrolyzed. The continuous reaction of the thiol group with DTNB, 5,5′-dithiobis(2-nitrobenzoic acid), produces the yellow color detected at 412 nm. The series of reactions are depicted below:

H_2O-$(CH_3)_3N^+(CH_2)_2SCOCH_3$ acetylthiocholine

↓ Acetylcholinesterase + Pseudocholinesterase

$(CH_3)_3N^+(CH_2)_2S^-$ + CH_3COO^- + $2H^+$
thiocholine acetate

$(CH_3)_3N^+(CH_2)_2S^-$ + RSSR (DTNB)
thiocholine

↓

$(CH_3)_3N^+(CH_2)_2SSR$ + RS^- (produces yellow color)

At assay time the samples were allowed to reach room temperature and the tubes inverted to resuspend the red blood cells. Hemolysis was not necessary since acetylcholinesterase is located on the red cell membrane. Two cuvettes were filled with 1.0 ml sample suspension and 10 μl of the reagent 0.01 M DTNB (39.6 mg/10 ml 0.1 M pH 7.0 phosphate buffer with 15 mg $NaHCO_3$) added. One cuvette served as the blank and the other as sample. Ten μl of the substrate, 0.075 M acetylthiocholine iodide, were added to the sample cuvette only, and changes in absorbance at 412 nm monitored over 6 minutes. The assay sensitivity is approximately ±4% of the mean. Total esterase activity is expressed as units/ml blood, where units are defined as moles hydrolyzed per min, and calculated as follows:

$$\frac{\text{delta absorbance/min} \times 1.02 \times 601}{13{,}600/10^3 \times 1 \times 1} = \text{moles/min/ml blood}$$

where,

1.02 = total volume in cuvette

601 = dilution factor

13,600 = absorbance coefficient

1 = light path length (cm)

1 = sample volume (ml)

Since ACh is hydrolyzed by both acetylcholinesterase and nonspecific cholinesterase, the enzyme rate determined with acetylthiocholine is considered a good estimate of the rate of ACh hydrolysis.

Plasma lactate levels were analyzed using a YSI-Model 27 Lactate Analyzer. One ml arterial and venous blood samples were collected into chilled test tubes, centrifuged under refrigeration at 10,000 rpm for 10 minutes and the plasma decanted. Twenty five μl of plasma was injected into the lactate analyzer where it passed through a membrane with immobilized enzyme l-lactate oxidase. This enzyme converted the lactate in the sample into hydrogen peroxide (H_2O_2). The H_2O_2 is oxidized at the platinum working electrode. The current flow in this electrode is linearly proportional to the local concentration of H_2O_2. The platinum electrode is maintained at an electrical potential of +0.70 volts with respect to a silver/silver chloride reference electrode. An operational amplifier measured the potential difference between the working and reference electrodes and forced sufficient current through a third electrode to hold it at +0.70 volts. The result was a signal current proportional to the lactate concentration, which was converted to a voltage, scaled

according to zero and calibration settings, and displayed on a digital meter (1 mV = 1 mg/dl lactate). The sensitivity of this assay is 2 mg/dl ± 2% relative precision.

Pyruvate was quantified by utilization of a SIGMA pyruvate assay kit. One ml arterial and venous blood samples were collected into chilled tubes containing 2.0 ml cold 8% perchloric acid (PCA), vortexed, centrifuged at 10,000 rpm for 10 min under refrigeration. The supernatant was removed and used for spectrophotometric determination (Beckman DU-65 Spectrophotometer) of pyruvate content. The pyruvate in the sample was converted into lactate via lactate dehydrogenase with spectrophotometric detection of nicotinamide adenine dinucleotide (NAD) according to the following reaction:

Pyruvate + NADH

↓ Lactate Dehydrogenase

Lactate + NAD

In the presence of excess NADH, all pyruvate is converted to lactate. The reduction of absorbance at 340 nm due to oxidation of NADH to NAD becomes a measure of the amount of pyruvate in the sample, i.e. the NAD is proportional to the initial pyruvate level. Sensitivity of this method is considered to be 0.1 mg/dl.

DATA ANALYSIS

Statistical analysis of the data utilized the ABstat program for paired t-tests, and unpaired or independent t-tests. Differences were considered significant at the $p<0.05$ level. Intragroup comparisons utilized paired t-tests. Comparisons between Sedentary and Exercise Trained groups utilized the independent t-test. Data represented as % change first underwent arcsin transformation for proportions (Snedecor, 1966), before appropriate statistical analysis (paired or independent t-tests) were applied.

RESULTS

TRAINING VERIFICATION

Heart rate values measured during pre- and post-training treadmill stress tests are presented in Table I. Stress test protocol involved varying speeds (kph), and treadmill gradient (%). Pre-treadmill stress test heart rates were significantly different from post-treadmill stress test heart rates, with the exception of 5 kph - 0% in the Sedentary group using a paired t-test analysis. The Exercise Trained group showed consistently lower heart rates than the Sedentary group post-training, with significant differences in heart rate at control, 5 kph - 0%, and 5 min recovery stages with independent t-test analysis. Control delta (pre - post heart rate) was significantly different between the two groups with independent t-test at $p=0.08$.

Venous lactate measurements from blood samples taken after pre- and post-training treadmill stress tests are presented in Table II. There was no difference between pre- and post-training venous lactate values in the Sedentary group. The Exercise Trained group exhibited a 30 % decrease in venous lactate with training (14.0 ± 3.1 mM vs. 10.3 ± 3.0 mM). The Exercise Trained group post-stress test venous lactate was significantly different from the Sedentary group. These lactate values are comparable to those values reported in other exercise studies in swine. Hastings et al. (1982) reported post-exercise venous lactate values in untrained pigs at 17.8 ± 1.6 mM; McKirnan et al. (1986) reported venous lactate values at maximal exercise in trained pigs before (19.8 ± 2.8 mM) and after training (12.9 ± 3.0 mM). Significance

was analyzed with an independent t-test between groups and a paired t-test within groups. The % change data underwent arcsin transformation before t-test analysis.

HEART WEIGHTS

After termination of the experiment, various regions of the excised heart were weighed. Exercise training had no effect on any of the following measurements (Sedentary vs. Exercise Trained): whole heart weight (163 ± 9 g vs. 159 ± 5 g) , left ventricular weight (101 ± 5 g vs. 102 ± 4 g), perfused tissue weight (50 ± 4 g vs. 53 ± 5 g), heart/body weight (4.2 ± 0.2 vs. 4.1 ± 0.1) and left ventricle/body weight (2.6 ± 0.1 vs. 2.6 ± 0.1) ratios. There was no evidence of hypertrophy with exercise training.

TOTAL CHOLINESTERASE

Total cholinesterase arterial and venous values for Sedentary and Exercise Trained groups are listed in Table III. These measurements were taken before the thoracotomy procedure. There were no differences between groups in arterial or venous values as analyzed with independent t-tests. These values are comparable with those reported by Ellman et al. (1961) in humans, where acetylcholinesterase specifically was 1.08 ± 0.16 moles/min/red blood cell.

TABLE I

TREADMILL STRESS TEST HEART RATE DATA

PROTOCOL	GROUP	PRE	POST *	Δ
CONTROL	SEDENTARY (9)	164 ±13	124 ±11	39 ±14
	EXERCISE (12)	169 ±11	103 † ±8	66 § ±12
5 KPH - 0%	SEDENTARY (9)	287 ±7	248 ±10	62 ±23
	EXERCISE (12)	277 ±10	210 † ±8	67 ±9
5 KPH- 10%	SEDENTARY (9)	311 ±7	267 ±8	45 ±8
	EXERCISE (12)	302 ±7	250 ±7	52 ±5
7 KPH - 10%	SEDENTARY (9)	317 ±8	278 ±8	67 ±29
	EXERCISE (12)	318 ±5	268 ±5	50 ±5
5 MIN RECOVERY	SEDENTARY (9)	187 ±6	165 ±8	21 ±12
	EXERCISE (12)	177 ±8	145 † ±7	30 ±8

Values are expressed as beats/min; mean ± S.E.M.
PRE = test before 10 week training or sedentary period
POST = test after 10 week training or sedentary period
Δ = PRE - POST values
* Significantly different from Pre values (except Sedentary 5 KPH-0%); $p<0.05$
† Significantly different from Sedentary; $p<0.05$
§ Significantly different from Sedentary; $p=0.08$

TABLE II

TREADMILL STRESS TEST VENOUS LACTATE DATA

GROUP	PRE	POST
SEDENTARY (5)	18.7 ±2.8	20.2 ±3.5
EXERCISE (5)	14.0 ±3.1	10.3 * † ±3.0

Values are expressed as mM; mean ± S.E.M.
PRE = test before 10 week training or sedentary period
POST = test after 10 week training or sedentary period
* Significantly different from Exercise-Pre; $p<0.05$
† Significantly different from Sedentary; $p<0.05$

TABLE III

TOTAL CHOLINESTERASE ACTIVITY IN BLOOD

GROUP	ARTERIAL	VENOUS
SEDENTARY (6)	1.91 ±0.27	1.72 ±0.29
EXERCISE (6)	1.70 ±0.15	1.60 ±0.11

Values are expressed as units/ml blood; mean ± S.E.M.

INTRACORONARY ACETYLCHOLINE

Intracoronary Bolus Injections

Control and Simulated Exercise Dose Responses

Percent reductions in LAD coronary artery blood flow following intracoronary bolus injections of ACh are illustrated in Figures 1-4. Blood flow was reduced in a dose-dependent manner. Significant differences in percent decrease in coronary blood flow were determined by arcsin transformation, followed by t-test analysis. Paired t-tests were used to determine response differences between control and NE infusion ACh bolus injections within groups. Independent t-tests were used to determine differences between groups at the various ACh doses at control and during NE infusion.

Figures 1 and 2 illustrate the % reduction in coronary blood flow as a result of intracoronary bolus injections of ACh (0.5 - 4.0 μg) at rest, referred to as control injections (Figure 1), and during increased cardiac work (Figure 2). There were no differences in the % reduction in coronary blood flow between groups at each of the ACh doses at rest, or during increased cardiac work.

The ACh dose responses of the Sedentary group at control and during simulated exercise with intravenous NE are compared in Figure 3. The % decrease in coronary blood flow during NE infusion injections was significantly less at all doses than the reduction responses for control injections. Exercise Trained group comparison of the ACh dose responses at control and during NE infusion are

illustrated in Figure 4. The lower ACh doses of 1.0 and 2.0 μg were significantly less during NE infusion injections than control injections.

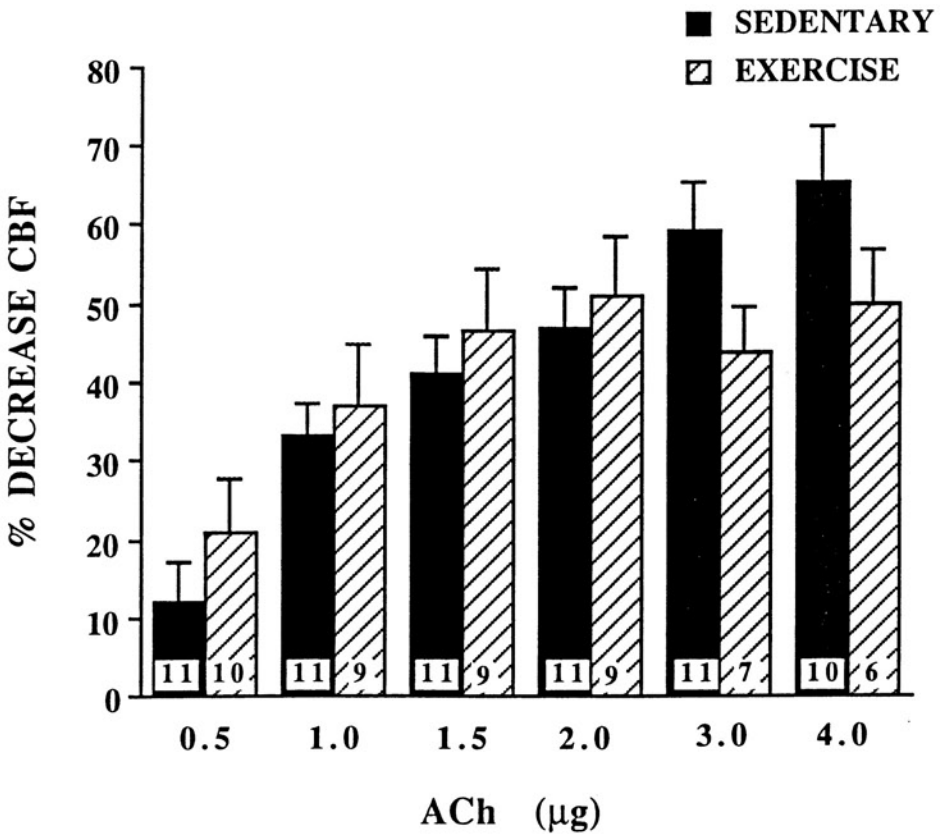

Figure 1. The responses to IC bolus injections of ACh (0.5 - 4.0 μg) administered at rest are reported as % decrease in coronary blood flow. The number of animals per dose is designated at the bottom of the individual bars. The control coronary blood flows (mean $\pm$ SEM, ml/min/100g) before ACh injections were (Sedentary, Exercise): 119 ± 8, 120 ± 19 for 0.5 μg; 127 ± 13, 130 ± 19 for 1.0 μg; 129 ± 12, 129 ± 20 for 1.5 μg; 125 ± 112, 130 ± 20 for 2.0 μg; 129 ± 14, 152 ± 26 for 3.0 μg; 114 ± 8, 150 ± 26 for 4.0 μg.

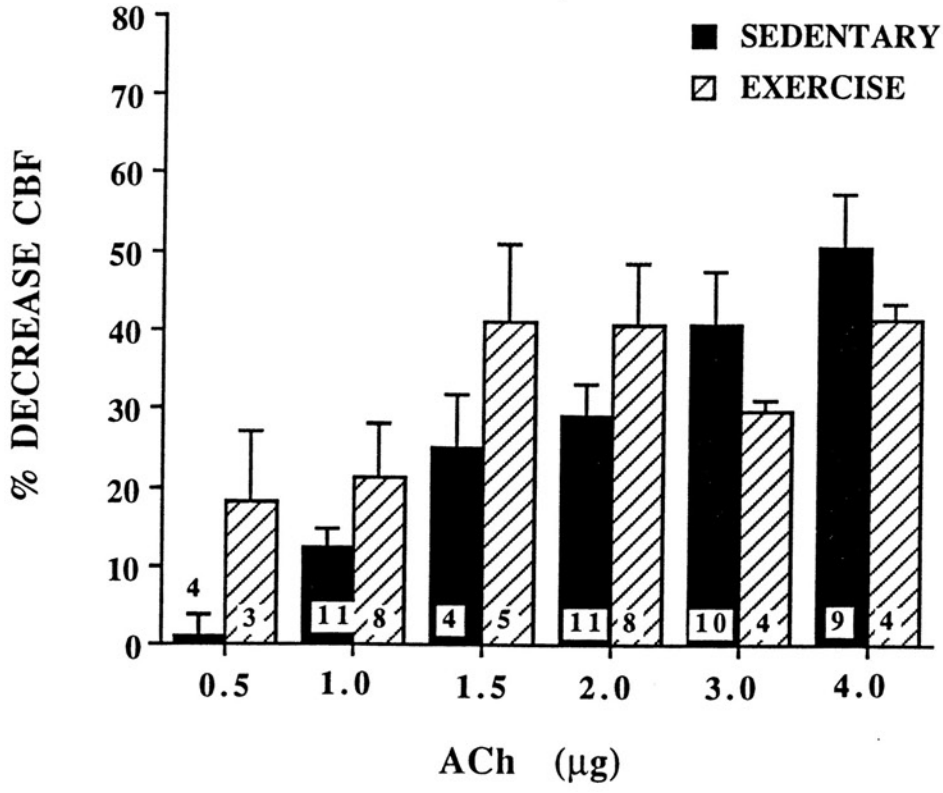

Figure 2. The responses to IC bolus injections of ACh (0.5 - 4.0 μg) administered during simulated exercise with IV NE infusion (60-70% increase PRP) are reported as % decrease in coronary blood flow. The number of animals per dose is designated at the bottom of the individual bars. The control coronary blood flows (mean $\pm$ SEM, ml/min/100g) before ACh injections were (Sedentary, Exercise): 224 ± 44, 143 ± 6 for 0.5 μg; 183 ± 25, 213 ± 23 for 1.0 μg; 229 ± 52, 188 ± 30 for 1.5 μg; 187 ± 24, 215 ± 24 for 2.0 μg; 175 ± 20, 238 ± 32 for 3.0 μg; 156 ± 15, 229 ± 28 for 4.0 μg.

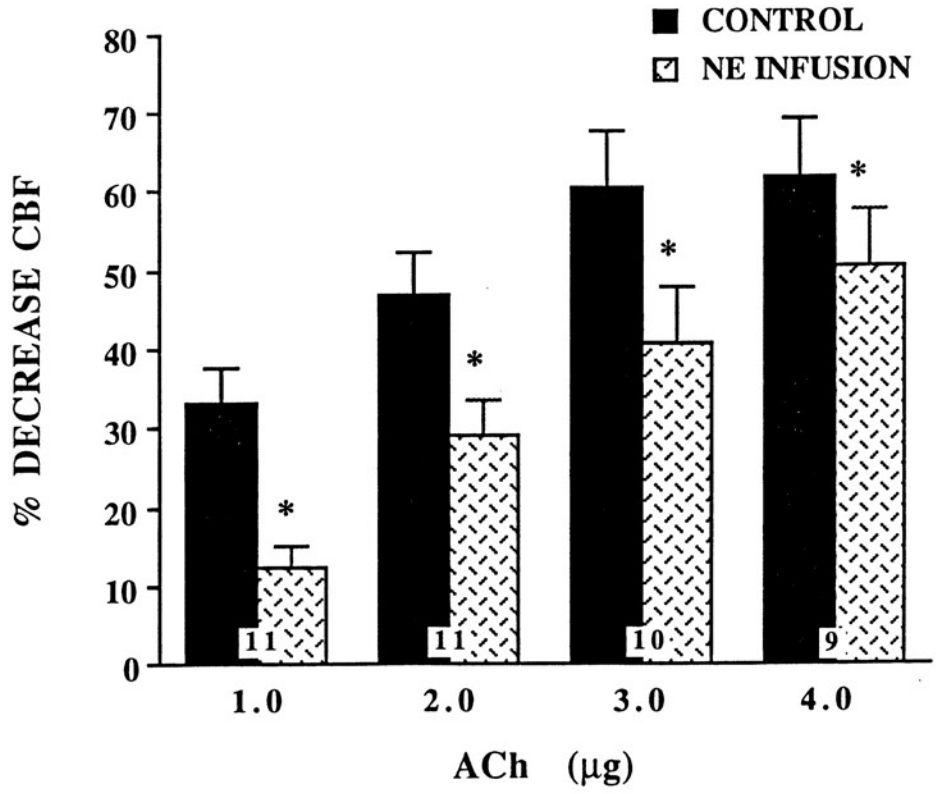

Figure 3. The responses of the Sedentary group to IC bolus injections of ACh at rest (control) and during simulated exercise with IV NE infusion are reported as % decrease in coronary blood flow. The number of animals per dose is designated at the bottom of the individual bars. The control coronary blood flows (mean ± SEM, ml/min/100g) before ACh injections were (Control, NE Infusion): 127±13, 183±25 for 1.0 μg; 125±12, 187±24 for 2.0 μg; 129±14, 175±20 for 3.0 μg; 114±8, 156±15 for 4.0 μg. Paired data comparison of % decrease in coronary blood flow at 1.0 - 4.0 μg ACh resulted in significant differences (*) at all doses at $p<0.05$.

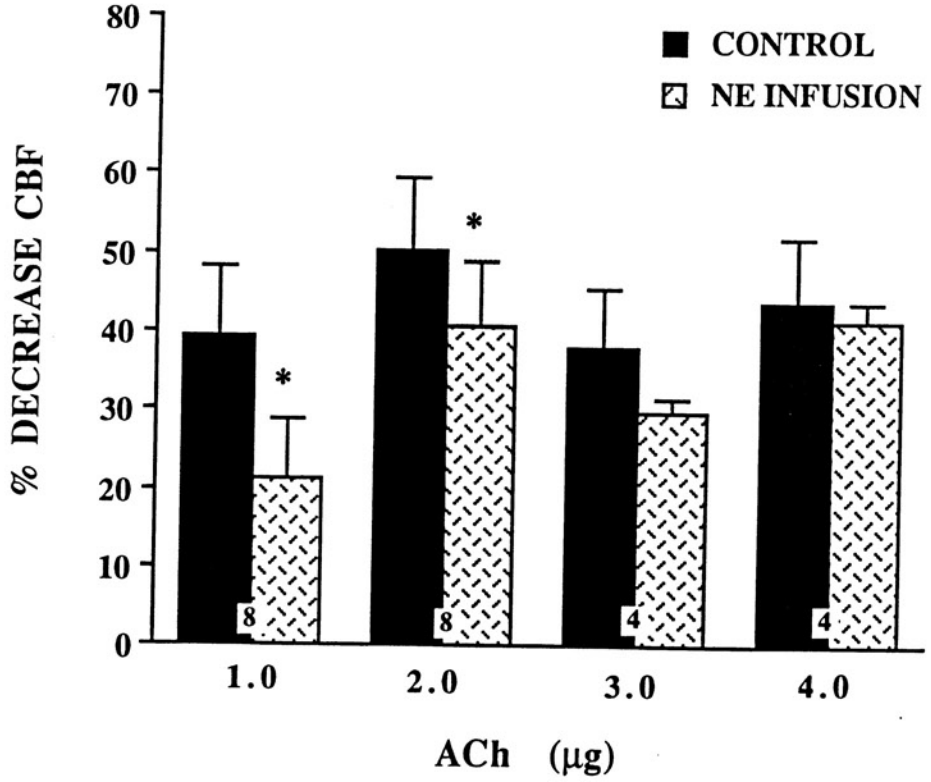

Figure 4. The responses of the Exercise Trained group to IC bolus injections of ACh at rest (control) and during simulated exercise with IV NE infusion are reported as % decrease in coronary blood flow. The number of animals per dose is designated at the bottom of the individual bars. The control coronary blood flows (mean ± SEM, ml/min/100g) before ACh injections were (Control, NE Infusion): 130±19, 213±23 for 1.0 μg; 130±20, 215±24 for 2.0 μg; 152±26, 238±32 for 3.0 μg; 150±26, 229±28 for 4.0 μg. Paired data comparison of % decrease in coronary blood flow at 1.0 - 4.0 μg ACh resulted in significant differences (*) at 1.0 and 2.0 μg ACh doses at $p<0.05$.

3.0 μg Comparisons

Comparisons between the Sedentary and Exercise Trained group's reactivity to a 3.0 μg ACh bolus injections at rest or control, during simulated exercise with NE infusion, 1 minute after cessation of exercise (post-NE infusion), and after muscarinic receptor blockade with intracoronary atropine are summarized in Tables IV, V, and VI.

Table IV represents the calculated μM ACh concentration achieved in the LAD with a 3.0 μg ACh injection, and the corresponding reduction in coronary blood flow. In order to calculate ACh concentration, one must take into account the ACh dose injected (3.0 μg), the rate of injection (0.025 min), the coronary blood flow at the injection site, and the molecular weight of ACh (181.7 g/mole). Below is the equation used for calculation of μM ACh:

$$\frac{3.0\ \mu g}{0.025\ min} \times \frac{1}{CBF}\frac{min}{ml} \times \frac{\mu mol}{181.7\ \mu g} \times \frac{10^3\ ml}{liter}$$

For each bolus injection group, there was no difference in the percent reduction in coronary blood flow between the Sedentary and Exercise Trained groups using independent t-test analysis after arcsin transformation. The concentration of ACh injected, with respect to the dilutionary effects of coronary blood flow, was comparable across both groups at control and during NE infusion. The ACh concentration at the post-exercise (post-NE infusion) bolus injections and post-atropine injections were significantly different. The ACh concentration in the Sedentary group was significantly different from the Exercise Trained group, but resulted in a comparable reduction in coronary blood flow. Figure 5 illustrates the

comparison of μM ACh and % decrease in coronary blood flow at the four 3.0 μg ACh trials.

Muscarinic receptor blockade had significant effects on the reduction in coronary blood flow as demonstrated in Table IV and Figure 5. ACh-mediated coronary vasoconstriction was inhibited by the muscarinic receptor antagonist atropine. There was no reduction in coronary blood flow calculated after intracoronary administration of atropine in either the Sedentary or the Exercise Trained group. The post-atropine ACh challenge was significantly different from all other calculated percent reductions in both Sedentary and Exercise Trained groups using paired t-test analysis after arcsin transformation.

Hemodynamic parameters including heart rate, mean arterial blood pressure, peak intraventricular pressure, pressure-rate product, contractility (dP/dt), coronary blood flow and coronary vascular resistance, are represented in Tables V and VI. There were no differences in these parameters between the Sedentary and Exercise Trained groups for each 3.0 μg bolus injection.

TABLE IV

ACh CONCENTRATION AND CORONARY BLOOD FLOW REDUCTION CALCULATIONS FOR 3.0 μg DOSES OF ACh

PROTOCOL 3.0 μg ACh	GROUP	ACh μM	% ↓ CBF
CONTROL	SEDENTARY (11)	12.2 ±1.5	59.2 † ±6.2
	EXERCISE (7)	8.5 ±0.2	48.3 ‡ ±6.2
NE INFUSION	SEDENTARY (10)	9.4 ±1.3	40.6 † § ±6.8
	EXERCISE (5)	5.4 ±0.6	38.3 ‡ ±7.7
POST NE INFUSION	SEDENTARY (5)	19.5 ±3.4	41.8 † § ±7.6
	EXERCISE (3)	7.2 * ±0.6	40.4 ‡ ±9.3
POST ATROPINE	SEDENTARY (8)	19.8 ±3.3	-0.5 § ±0.5
	EXERCISE (7)	9.3 * ±1.6	-0.5 ±0.4

Values are mean ± S.E.M.

* Significantly different from Sedentary; $p<0.05$

† Significantly different from Sedentary Post-Atropine; $p<0.05$

‡ Significantly different from Exercise Post-Atropine; $p<0.05$

§ Significantly different from Sedentary Control; $p<0.05$

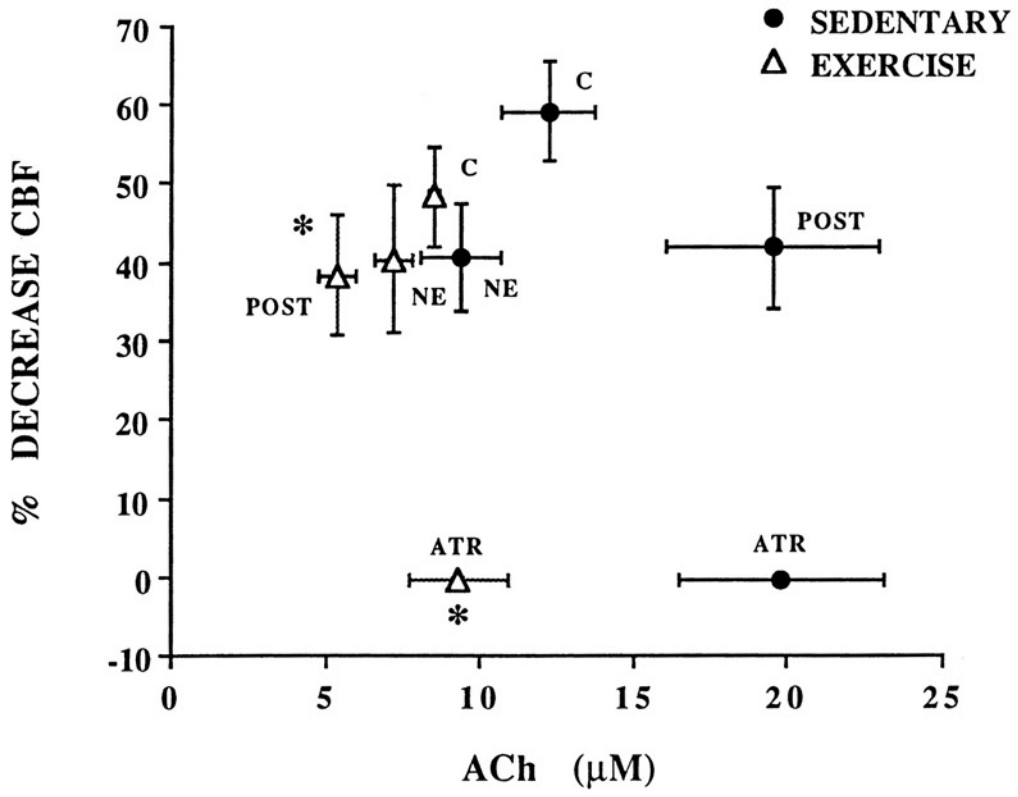

Figure 5. ACh concentrations (μM) calculated from the 3.0 μg ACh IC bolus injections administered at control (C), during simulated exercise with intravenous NE infusion (NE), 1 min post-NE infusion (POST), and after muscarinic receptor blockade with intracoronary atropine injection (ATR) are plotted against the resulting % decrease in coronary blood flow. Comparisons of μM ACh and % decrease CBF were made between Sedentary and Exercise Trained groups for each injection time. (*) represents significant differences in ACh concentration between Sedentary and Exercise Trained groups ($p<0.05$).

TABLE V

COMPARISON OF HEMODYNAMIC PARAMETERS
FOR 3.0 μg DOSES OF ACh

PROTOCOL 3.0μg ACh	GROUP	HR beats /min	MABP mmHg	PIP mmHg	PRP mmHg /min	dP/dt mmHg /sec
CONTROL	SEDENTARY (11)	120 ±6	103 ±6	112 ±6	13546 ±1043	1469 ±107
	EXERCISE (7)	124 ±5	93 ±4	104 ±5	13025 ±1041	1946 ±262
NE INFUSION	SEDENTARY (10)	133 ±8	131 ±6	149 ±9	19792 ±1799	4486 ±603
	EXERCISE (5)	138 ±6	120 ±8	136 ±10	18639 ±1459	5050 ±636
POST NE INFUSION	SEDENTARY (5)	120 ±7	78 ±11	92 ±12	11288 ±1869	1646 ±362
	EXERCISE (3)	140 ±10	88 ±7	97 ±7	13769 ±1799	2416 ±522
POST ATROPINE	SEDENTARY (8)	130 ±7	106 ±7	117 ±8	15155 ±1354	2030 ±297
	EXERCISE (7)	118 ±6	99 ±4	109 ±5	13028 ±1240	2284 ±440

Values are mean ± S.E.M.

TABLE VI

COMPARISON OF CORONARY BLOOD FLOW & RESISTANCE FOR 3.0 μg DOSES OF ACh

PROTOCOL 3.0μg ACh	GROUP	CBF ml/min/100g	CVR mmHg/ml/min/100g
CONTROL	SEDENTARY (11)	52 ±10	3.53 ±0.96
	EXERCISE (7)	83 ±20	1.72 ±0.41
NE INFUSION	SEDENTARY (10)	109 ±20	2.29 ±0.91
	EXERCISE (5)	145 ±29	1.13 ±0.33
POST NE INFUSION	SEDENTARY (5)	61 ±13	1.77 ±0.52
	EXERCISE (3)	120 ±31	0.95 ±0.27
POST ATROPINE	SEDENTARY (8)	111 ±19	1.11 ±0.15
	EXERCISE (7)	131 ±22	1.00 ±0.23

Values are mean ± S.E.M.

Intracoronary Infusions

Table VII summarizes the calculated μM ACh concentration in the LAD required to achieve a 10% and 30% reduction in coronary blood flow. In order to calculate ACh concentration, one must take into account the ACh solution infused, coronary blood flow at the injection site, the molecular weight of ACh (181.7 g/mole), and the pump rate of infusion. Below is the equation used for calculation of μM ACh:

$$\text{ACh}\ \frac{\mu g}{ml} \times \text{CBF}\ \frac{ml}{min} \times \frac{\mu mol}{181.7\ \mu g} \times \frac{min}{ml} \times \frac{10^3\ ml}{liter}$$

There was no difference in the ACh concentration required for a 10% reduction in coronary blood flow between the Sedentary and Exercise Trained animals, approximately 0.4 μM in both groups. However, when a 30% reduction in coronary blood flow was achieved with intracoronary ACh infusion, there was a significant difference between the Sedentary and Exercise Trained group in the amount required (independent t-test analysis).

Hemodynamic parameters measured during intracoronary infusion are summarized in Table VIII and Table IX. Left ventricular dP/dt measured during ACh infusion at 30% reduction in coronary blood flow was significantly different from saline infusion dP/dt, using paired t-test analysis, in both Sedentary and Exercise Trained groups. Coronary blood flow at the 30% reduction was signficantly lower in both Sedentary and Exercise Trained groups when compared to paired saline infusion and 10% reduction coronary blood flow values. In addition, the coronary blood flow in the Exercise Trained group at the 10% reduction was

significantly different from saline infusion coronary blood flow. In the Sedentary group, coronary vascular resistance at the 30% reduction in coronary blood flow was significantly different from control infusion. In the Exercise Trained group, coronary vascular resistance was significantly different from that calculated during ACh infusion at 10% reduction in coronary blood flow.

Table X and Table XI summarize the myocardial blood gas and oxygen parameters, including calculated oxygen extraction, $(A\text{-}V)O_2$, and myocardial oxygen consumption (MVO_2) values. There were no significant differences between Sedentary and Exercise Trained groups in any of these parameters.

TABLE VII

CALCULATED ACh CONCENTRATION
DURING IC ACh INFUSIONS

PROTOCOL	GROUP	μM ACh
ACh INFUSION 10% ↓ CBF	SEDENTARY (8)	0.455 ±0.092
	EXERCISE (8)	0.397 ±0.076
ACh INFUSION 30% ↓ CBF	SEDENTARY (8)	1.507 ±0.270
	EXERCISE (6)	0.648 * ±0.115

Values are mean ± S.E.M.
* Significantly different from Sedentary; $p<0.05$

TABLE VIII

HEMODYNAMIC PARAMETERS DURING IC INFUSIONS

PROTOCOL	GROUP	HR beats /min	MABP mmHg	PIP mmHg	PRP mmHg /min	dP/dt mmHg /sec
SALINE INFUSION	SEDENTARY (11)	121 ±6	96 ±7	107 ±7	12957 ±1176	1786 ±216
	EXERCISE (9)	118 ±4	92 ±3	104 ±3	12269 ±716	2321 ±388
ACh INFUSION 10% ↓ CBF	SEDENTARY (8)	111 ±6	101 ±9	110 ±8	12151 ±1132	1528 ±186
	EXERCISE (8)	117 ±6	93 ±3	104 ±5	12264 ±1045	1963 ±298
ACh INFUSION 30% ↓ CBF	SEDENTARY (8)	123 ±8	93 ±11	105 ±11	12515 ±1311	1319 * ±154
	EXERCISE (6)	117 ±7	89 ±4	98 ±4	11507 ±1019	1665 * ±239

Values are mean ± S.E.M.

* Significantly different from Saline Infusion; $p<0.05$

TABLE IX

CORONARY BLOOD FLOW & RESISTANCE
DURING IC INFUSIONS

PROTOCOL	GROUP	CBF ml/min/100g	CVR mmHg/ml/min/100g
SALINE INFUSION	SEDENTARY (11)	113 ±12	0.95 ±0.12
	EXERCISE (9)	126 ±22	0.98 ±0.18
ACh INFUSION 10% ↓ CBF	SEDENTARY (8)	95 ±11	1.16 ±0.15
	EXERCISE (8)	107 * ±23	1.42 ±0.37
ACh INFUSION 30% ↓ CBF	SEDENTARY (8)	64 * † ±6	1.51 * ±0.19
	EXERCISE (6)	85 * † ±20	1.76 † ±0.56

Values are mean ± S.E.M.
* Significantly different from Saline Infusion; $p<0.05$
† Significantly different from ACh Infusion, 10% ↓ CBF; $p<0.05$

TABLE X

MYOCARDIAL BLOOD GASES DURING IC INFUSIONS

PROTOCOL	GROUP	pH		pCO_2 mmHg		pO_2 mmHg	
		A	V	A	V	A	V
SALINE INFUSION	SEDENTARY (11)	7.521 ±.017	7.464 ±.015	33.6 ±2.0	40.8 ±2.4	97.7 ±7.5	15.1 ±1.2
	EXERCISE (9)	7.521 ±.025	7.469 ±.021	34.2 ±2.3	41.7 ±2.3	84.9 ±5.6	14.0 ±1.1
ACh INFUSION 10% ↓ CBF	SEDENTARY (8)	7.529 ±.018	7.455 ±.017	31.7 ±1.9	42.3 ±2.0	96.0 ±9.0	15.1 ±1.1
	EXERCISE (7)	7.537 ±.026	7.453 ±.021	33.0 ±2.4	44.1 ±1.9	80.7 ±4.9	13.8 ±1.3
ACh INFUSION 30% ↓ CBF	SEDENTARY (7)	7.513 ±.019	7.418 ±.018	33.7 ±2.3	44.8 ±2.5	101.1 ±11.4	15.4 ±1.2
	EXERCISE (6)	7.534 ±.030	7.449 ±.025	31.8 ±2.3	44.5 ±2.6	82.6 ±5.0	14.6 ±1.3

Values are mean ± S.E.M.

TABLE XI

MYOCARDIAL OXYGEN PARAMETERS DURING IC INFUSIONS

PROTOCOL	GROUP	OXYGEN CONTENT vol %		$(A-V)O_2$ vol %	MVO_2 ml O_2/ min/100g
		A	V		
SALINE INFUSION	SEDENTARY (11)	14.0 ±0.6	2.0 ±0.3	11.9 ±0.6	13.2 ±1.1
	EXERCISE (9)	14.0 ±0.7	2.1 ±0.2	11.9 ±0.7	13.2 ±1.9
ACh INFUSION 10% ↓ CBF	SEDENTARY (8)	13.2 ±0.5	1.7 ±0.2	11.7 ±0.5	10.7 ±1.1
	EXERCISE (7)	13.9 ±0.7	2.1 ±0.2	11.8 ±0.7	13.0 ±2.2
ACh INFUSION 30% ↓ CBF	SEDENTARY (7)	13.1 ±0.6	1.6 ±0.2	11.5 ±0.7	8.7 ±1.1
	EXERCISE (6)	13.9 ±0.8	2.2 ±0.3	11.7 ±0.8	9.1 ±1.8

Values are mean ± S.E.M.

NOREPINEPHRINE INFUSION/SIMULATED EXERCISE

Table XII demonstrates the concentration of NE that was infused intravenously to achieve a 60 - 70% increase in pressure-rate product to simulate an acute bout of exercise. The NE concentration took into account the NE solution infused, the infusion pump rate, and the animal body weight (kg) for standardization. The equation used in this calculation is as follows:

$$\frac{\frac{\mu g\ NE}{ml} \times \frac{ml}{min}}{kg\ body\ weight}$$

There was a significant difference between groups in the amount of norepinephrine required to achieve this increase in cardiac work using an independent t-test.

Hemodynamic parameters in Table XIII and Table XIV demonstrate that there are significant differences in MABP, PIP, PRP, dP/dt, and CBF at the increased workload when compared to baseline values in both the Sedentary and Exercise Trained groups using paired t-test analysis. In addition, Exercise Trained dP/dt was significantly different from Sedentary both before and during NE infusion (independent t-test). In the Exercise Trained group there were significant differences in CBF and CVR during NE infusion, and in the Sedentary group there was a significant difference in CBF when compared to pre-NE infusion values. Although there was a tendency for coronary blood flows to be higher in the Exercise Trained pigs, the difference did not reach statistical significance.

Myocardial blood gas and oxygen parameter data before and during NE infusion are presented in Table XV and Table XVI. Venous oxygen content during NE infusion in the Sedentary group was significantly different from pre-NE infusion with paired t-test analysis. Myocardial oxygen consumption during NE infusion in the Exercise Trained group was significantly different from pre-NE infusion with paired t-test analysis. It was also significantly different from oxygen consumption in the Sedentary group at the increased cardiac workload with independent t-test analysis.

Figure 6 illustrates the differences in myocardial oxygen consumption and coronary blood flow before and during NE infusion between the Sedentary and Exercise Trained groups.

TABLE XII

NE CONCENTRATION CALCULATED
DURING EXERCISE SIMULATION

PROTOCOL	GROUP	% ↑ PRP	[NE] μg/min/kg
NE INFUSION (Intravenous)	SEDENTARY (6)	67 ±2	0.35 ±0.07
	EXERCISE (5)	62 ±2	0.73 * ±0.15

Values are mean ± S.E.M.
* Significantly different from Sedentary; $p<0.05$

TABLE XIII

HEMODYNAMIC PARAMETERS BEFORE AND DURING SIMULATED EXERCISE WITH IV NE INFUSION

PROTOCOL	GROUP	HR beats /min	MABP mmHg	PIP mmHg	PRP mmHg /min	dP/dt mmHg /sec
PRE-NE INFUSION	SEDENTARY (11)	117 ±6	103 ±6	114 ±5	13285 ±808	1919 ±133
	EXERCISE (8)	118 ±6	99 ±4	109 ±4	12981 ±981	2635 * ±432
NE INFUSION	SEDENTARY (7)	121 ±5	148 † ±9	173 † ±11	20777 † ±1438	5344 † ±444
	EXERCISE (5)	133 ±6	137 ‡ ±6	168 ‡ ±6	22403 ‡ ±1262	6707 *‡ ±478

Values are mean ± S.E.M.
* Significantly different from Sedentary; $p<0.05$
† Significantly different from Sedentary Pre-NE Infusion; $p<0.05$
‡ Significantly different from Exercise Pre-NE Infusion; $p<0.05$

TABLE XIV

CORONARY BLOOD FLOW & RESISTANCE BEFORE AND DURING SIMULATED EXERCISE WITH IV NE INFUSION

PROTOCOL	GROUP	CBF ml/min/100g	CVR mmHg/ml/min/100g
PRE-NE INFUSION	SEDENTARY (11)	112 ±14	1.04 ±0.10
	EXERCISE (8)	138 ±23	0.91 ±0.16
NE INFUSION	SEDENTARY (7)	171 † ±30	1.05 ±0.17
	EXERCISE (5)	225 ‡ ±30	0.66 ‡ ±0.09

Values are mean ± S.E.M.
† Significantly different from Sedentary Pre-NE Infusion; $p<0.05$
‡ Significantly different from Exercise Pre-NE Infusion; $p<0.05$

TABLE XV

MYOCARDIAL BLOOD GASES BEFORE AND DURING SIMULATED EXERCISE WITH IV NE INFUSION

PROTOCOL	GROUP	pH		pCO_2 mmHg		pO_2 mmHg	
		A	V	A	V	A	V
PRE-NE INFUSION	SEDENTARY (11)	7.533 ±.019	7.469 ±.020	33.8 ±2.3	43.3 ±3.3	99.1 ±4.3	16.1 ±1.6
	EXERCISE (8)	7.527 ±.023	7.471 ±.021	34.2 ±2.5	43.5 ±2.9	88.0 ±7.6	15.0 ±1.5
NE INFUSION	SEDENTARY (7)	7.519 ±.019	7.460 ±.020	34.4 ±2.0	44.0 ±3.3	93.7 ±4.1	20.3 ±1.7
	EXERCISE (5)	7.519 ±.020	7.451 ±.017	34.8 ±2.3	45.9 ±2.4	81.5 ±7.3	18.3 ±1.3

Values are mean ± S.E.M.

TABLE XVI

MYOCARDIAL OXYGEN PARAMETERS BEFORE AND DURING SIMULATED EXERCISE WITH IV NE INFUSION

PROTOCOL	GROUP	OXYGEN CONTENT vol %		$(A-V)O_2$ vol %	MVO_2 ml O_2/ min/100g
		A	V		
PRE-NE INFUSION	SEDENTARY (7)	13.0 ±0.7	2.1 ±0.3	11.0 ±0.8	12.9 ±1.9
	EXERCISE (7)	14.0 ±0.6	2.2 ±0.4	11.8 ±0.7	17.1 ±1.4
NE INFUSION	SEDENTARY (7)	14.3 ±0.7	3.7 † ±0.5	10.6 ±0.7	17.3 ±2.4
	EXERCISE (5)	15.1 ±0.5	3.4 ±0.5	11.7 ±0.9	25.9 *‡ ±3.3

Values are mean ± S.E.M.

* Significantly different from Sedentary; $p<0.05$

† Significantly different from Sedentary-Pre; $p<0.05$

‡ Significantly different from Exercise-Pre; $p<0.05$

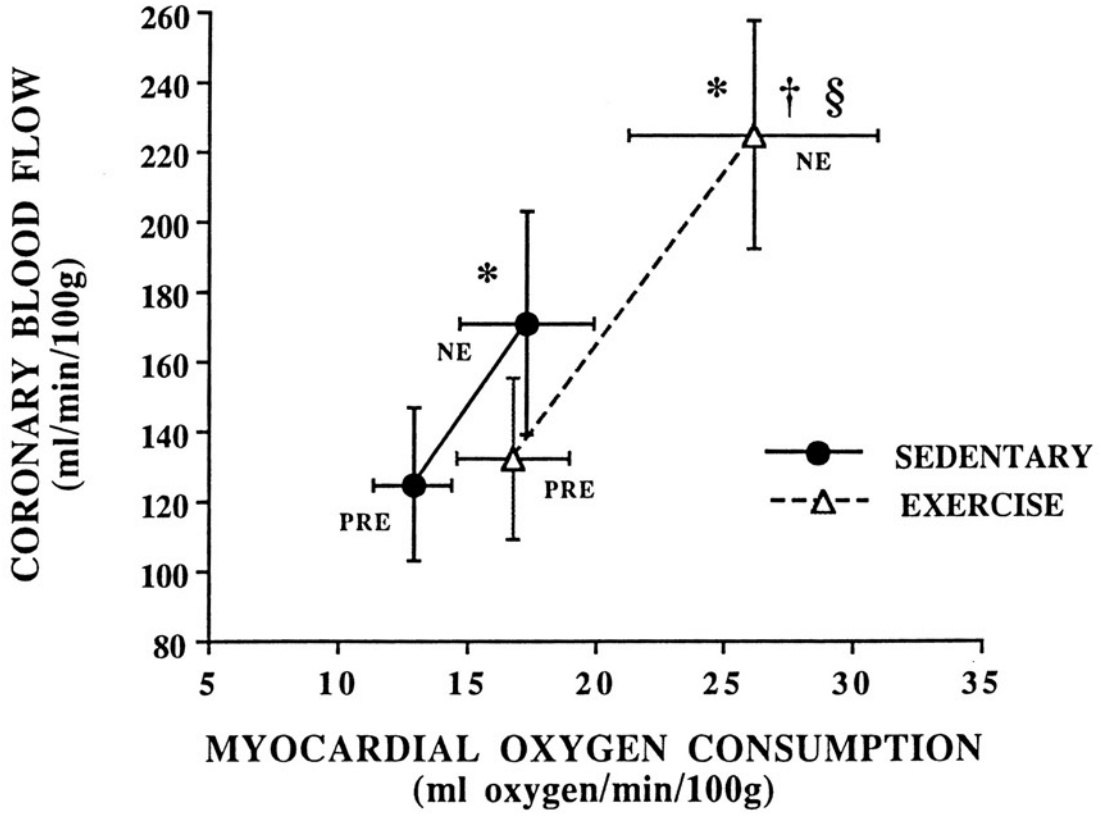

Figure 6. Myocardial oxygen consumption is plotted against coronary blood flow before (PRE), and during simulated exercise with intravenous NE infusion (NE) at a workload of 60-70% increase in pressure-rate-product. (*) represents significant differences between PRE and NE coronary blood flow in both groups. (†) represents significant difference between PRE and NE MVO_2 in the Exercise Trained group, and (§) represents significant difference in NE MVO_2 between Sedentary and Exercise Trained groups at $p<0.05$. The number of animals per group was Sedentary (7) and Exercise (5).

MUSCARINIC RECEPTOR BLOCKADE WITH ATROPINE

Hemodynamic parameters measured at control, and after muscarinic receptor blockade via intracoronary atropine bolus injection in the LAD coronary artery, are shown in Tables XVII and XVIII. Control MABP in the Exercise Trained group was significantly different from Sedentary using independent t-test. However, there were no differences in any of these parameters after atropine administration between groups or when compared to control. Myocardial blood gases and oxygen parameters are presented in Tables XIX and XX, respectively. All blood gas and oxygen parameters were comparable between groups.

TABLE XVII

HEMODYNAMIC PARAMETERS BEFORE AND AFTER MUSCARINIC RECEPTOR BLOCKADE WITH ATROPINE

PROTOCOL	GROUP	HR beats /min	MABP mmHg	PIP mmHg	PRP mmHg /min	dP/dt mmHg /sec
CONTROL	SEDENTARY (11)	122 ±6	110 ±6	119 ±6	14647 ±1156	2240 ±204
	EXERCISE (9)	118 ±6	93 * ±4	105 ±4	12373 ±888	2547 ±354
POST ATROPINE	SEDENTARY (9)	120 ±8	105 ±7	116 ±7	13981 ±1396	1972 ±275
	EXERCISE (7)	115 ±6	98 ±5	108 ±6	12557 ±1183	2373 ±466

Values are mean ± S.E.M.

* Significantly different from Sedentary; $p<0.05$

TABLE XVIII

CORONARY BLOOD FLOW & RESISTANCE BEFORE AND AFTER MUSCARINIC RECEPTOR BLOCKADE WITH ATROPINE

PROTOCOL	GROUP	CBF ml/min/100g	CVR mmHg/ml/min/100g
CONTROL	SEDENTARY (11)	120 ±11	0.97 ±0.08
	EXERCISE (9)	139 ±18	0.76 ±0.08
POST ATROPINE	SEDENTARY (9)	102 ±15	1.16 ±0.13
	EXERCISE (7)	123 ±22	1.14 ±0.31

Values are mean ± S.E.M.

TABLE XIX

MYOCARDIAL BLOOD GASES BEFORE AND AFTER MUSCARINIC RECEPTOR BLOCKADE WITH ATROPINE

PROTOCOL	GROUP	pH		pCO_2 mmHg		pO_2 mmHg	
		A	V	A	V	A	V
CONTROL	SEDENTARY (11)	7.531 ±.012	7.467 ±.013	33.4 ±1.4	42.9 ±1.9	102.1 ±6.2	15.2 ±1.0
	EXERCISE (9)	7.516 ±.027	7.464 ±.027	34.5 ±2.5	44.3 ±3.3	89.4 ±5.1	17.8 ±2.8
POST ATROPINE	SEDENTARY (9)	7.517 ±.019	7.457 ±.020	33.6 ±1.8	43.2 ±3.1	94.2 ±8.4	15.7 ±1.3
	EXERCISE (7)	7.528 ±.026	7.463 ±.024	33.5 ±2.1	45.3 ±2.6	84.4 ±7.3	14.7 ±1.1

Values are mean ± S.E.M.

TABLE XX

MYOCARDIAL OXYGEN PARAMETERS BEFORE AND AFTER MUSCARINIC RECEPTOR BLOCKADE WITH ATROPINE

PROTOCOL	GROUP	OXYGEN CONTENT vol %		$(A\text{-}V)O_2$ vol %	MVO_2 ml O_2/ min/100g
		A	V		
CONTROL	SEDENTARY (11)	13.5 ±0.5	2.2 ±0.2	11.3 ±0.5	13.4 ±1.0
	EXERCISE (9)	13.9 ±0.5	3.1 ±1.0	10.6 ±1.0	16.0 ±2.3
POST ATROPINE	SEDENTARY (9)	12.8 ±0.8	2.0 ±0.2	10.8 ±0.9	10.6 ±1.4
	EXERCISE (7)	13.8 ±0.5	2.5 ±0.3	11.3 ±0.5	13.5 ±2.1

Values are mean ± S.E.M.

MYOCARDIAL METABOLIC PARAMETERS

Catecholamine measurements from blood samples taken before the thoracotomy procedure, and designated as control values, are presented in Table XXI. There was a significant difference in control arterial norepinephrine concentrations between Sedentary and Exercise Trained using independent t-test analysis.

Table XXII shows that lactate consumption in the Exercise Trained group was significantly higher than the Sedentary group during NE infusion with independent t-test analysis. In the Exercise Trained group, lactate extraction (A-V) and consumption were significantly different when comparing NE infusion with pre-NE infusion. Post-atropine lactate extraction and consumption comparison with control reveals significant differences in both Sedentary and Exercise Trained groups. Data was paired and analyzed with a paired t-test.

Lactate data during intracoronary saline and ACh infusions are demonstrated in Table XXIII. Venous lactate was significantly different from arterial lactate in the Exercise group during saline infusion, and both ACh infusions. In the Sedentary group arterial lactate, lactate extraction, and lactate consumption during both ACh infusions, and venous lactate at the 30% reduction infusion, were significantly different from saline infusion values. In addition, the 30% reduction in CBF infusion showed significant differences from the 10% reduction in CBF in venous lactate, A-V, and lactate consumption in the Sedentary group. In the Exercise Trained group there were significant differences between arterial lactate, and lactate consumption

during saline infusion and 10% reduction CBF infusion. Lactate extraction and consumption during the 30% reduction CBF infusion was also significantly different from saline infusion in the Exercise Trained group. The lactate consumption in the Exercise Trained group was higher than the Sedentary group at $p=0.08$. Figure 7 illustrates the lactate consumption data during IC infusions of saline and ACh in both Sedentary and Exercise Trained groups. Intracoronary infusion lactate data were analyzed with a paired t-test for comparisons between the infusions within each group. Independent t-tests were utilized for comparisons between groups.

Venous lactate/venous pyruvate values are presented in Table XXIV. The Exercise Trained group had consistently higher L/P ratios that the Sedentary group using independent t-test analysis. In the Sedentary group the L/P ratio during ACh infusion at 30% reduction CBF was significantly different from all other Sedentary values .

TABLE XXI

CATECHOLAMINE CONCENTRATIONS
BEFORE THORACOTOMY PROCEDURE

	NOREPINEPHRINE pg/ml plasma		EPINEPHRINE pg/ml plasma	
	A	V	A	V
SEDENTARY (7)	157 ±19	234 ±35	136 ±49	103 ±47
EXERCISE (6)	252 * ±40	299 ±72	182 ±66	105 ±36

Values are mean ± S.E.M.
* Significantly different from Sedentary; $p<0.05$

TABLE XXII

MYOCARDIAL LACTATE DATA FOR CONTROL CONDITIONS, DURING SIMULATED EXERCISE, AND AFTER MUSCARINIC RECEPTOR BLOCKADE

PROTOCOL	GROUP	A mMol	V mMol	A - V mMol	CONSUMPTION μmol/min/100g
CONTROL	SEDENTARY (7)	1.46 ±0.20	0.68 § ±0.15	0.77 ±0.14	92 ±20
	EXERCISE (7)	2.57 ±0.58	1.64 ±0.46	0.93 ±0.12	127 ±11
PRE-NE INFUSION	SEDENTARY (7)	1.30 ±0.16	0.62 § ±0.14	0.67 ±0.11	84 ±19
	EXERCISE (7)	2.20 ±0.45	1.29 ±0.34	0.91 ±0.12	127 ±16
NE INFUSION	SEDENTARY (7)	1.37 ±0.20	0.79 § ±0.17	0.59 ±0.06	97 ±19
	EXERCISE (7)	2.20 ±0.35	1.43 ±0.26	0.78 † ±0.11	166 * † ±24
POST ATROPINE	SEDENTARY (7)	1.37 ±0.19	0.87 ±0.21	0.51 ‡ ±0.14	54 ‡ ±16
	EXERCISE (7)	1.70 ±0.12	1.14 ±0.11	0.56 ‡ ±0.09	69 ‡ ±14

Values are mean ± S.E.M.

* Significantly different from Sedentary; $p<0.05$

† Significantly different from Exercise Pre-NE; $p<0.05$

‡ Significantly different from Control; $p<0.05$

§ Significantly different from Arterial; $p<0.05$

TABLE XXIII

MYOCARDIAL LACTATE DATA DURING IC INFUSIONS

PROTOCOL	GROUP	A mMol	V mMol	A - V mMol	CONSUMPTION μmol/min/100g
SALINE INFUSION	SEDENTARY (7)	1.72 ±0.26	1.01 ±0.24	0.72 ±0.14	78 ±16
	EXERCISE (7)	2.29 ±0.23	1.47 § ±0.17	0.80 ±0.18	104 ±18
ACh INFUSION 10% ↓ CBF	SEDENTARY (7)	1.51 * ±0.21	1.00 ±0.23	0.52 * ±0.14	50 * ±12
	EXERCISE (7)	1.81 * ±0.15	1.37 § ±0.12	0.44 ±0.17	47 * ±20
ACh INFUSION 30% ↓ CBF	SEDENTARY (7)	1.51 * ±0.24	1.49 * † ±0.28	0.02 * † ±0.28	-3 * † ±19
	EXERCISE (7)	1.96 ±0.23	1.61 § ±0.28	0.35 * ±0.09	29 * ±10

Values are mean ± S.E.M.

* Significantly different from Saline infusion; $p<0.05$

† Significantly different from ACh infusion - 10% ↓ CBF; $p<0.05$

§ Significantly different from Arterial; $p<0.05$

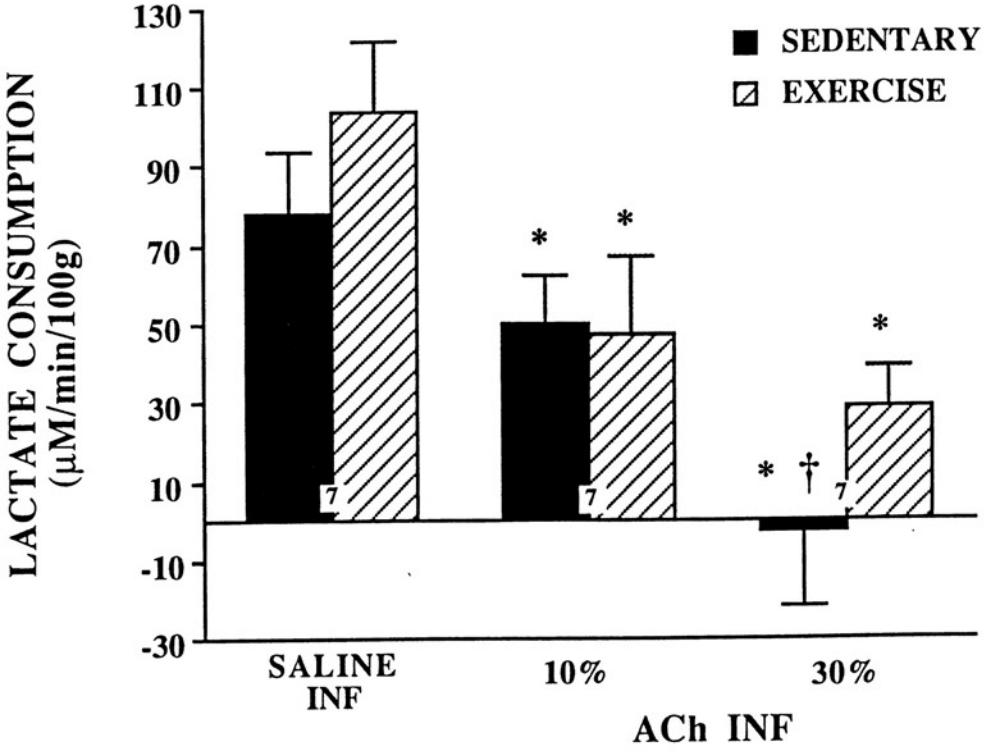

Figure 7. Comparison between lactate consumption during intracoronary infusions of saline (vehicle control) and ACh (10% and 30% reductions in coronary blood flow). (*) represents significant differences between lactate consumption during both ACh infusions vs. saline infusion in both Sedentary and Exercise Trained groups. (†) represents significant difference between lactate consumption in ACh infusions of 10% and 30% CBF reductions in the Sedentary group. (Paired t-tests at $p<0.05$).

TABLE XXIV

LACTATE/PYRUVATE RATIOS
FOR VENOUS BLOOD SAMPLES

PROTOCOL	SEDENTARY	EXERCISE
CONTROL	11 ±1	62 * ±10
PRE-NE INFUSION	10 ±1	60 * ±10
NE INFUSION	10 ±1	59 * ±13
SALINE INFUSION	10 ±1	53 * ±10
ACh INFUSION 10% ↓ CBF	10 ±1	48 * ±8
ACh INFUSION 30% ↓ CBF	15 † ±2	57 * ±11
POST ATROPINE	9 ±1	56 * ±12

Values are mean ± S.E.M.
* Significantly different from Sedentary; $p<0.05$
† Significantly different from all Sedentary values; $p<0.05$

DISCUSSION

There is increasing support for the enhancement of the parasympathetic nervous system as one of the major cardiovascular adaptations to endurance exercise training. It is considered to be primarily responsible for exercise bradycardia. In a review on the effects of exercise training on cardiac function, Barnard (1975) summarizes the possible mechanisms underlying the decrease in heart rate at rest and at any standardized submaximal workload. These include an increase in parasympathetic activity (increase in neural ACh), changes in the number and/or sensitivity of cholinergic receptors in the sinoatrial node, and an increase in non-neural ACh. These neural changes that alter the chronotropic state of the heart, are expected to change muscarinic-associated vascular responsiveness as well. The results of these studies support the hypothesis that endurance exercise training enhances the sensitivity of the coronary vasculature to ACh-mediated vasoconstriction in the porcine model. Responsiveness to intracoronary ACh bolus injections at rest, during simulated exercise, after cessation of exercise, and after muscarinic receptor blockade with atropine, indicate that the Exercise Trained swine were more sensitive to ACh in post-exercise conditions. During intracoronary ACh infusions, again the Exercise Trained swine demonstrated an increased sensitivity to ACh. Intracoronary atropine blocked ACh's actions, demonstrating involvement of the muscarinic receptor. Resting total cholinesterase, including acetylcholinesterase, was measured to determine if changes in exogenous ACh degradation were responsible for differences observed between animals. Hemodynamic parameters and coronary

metabolites were measured to provide information on other possible alterations in cardiovascular function and blood flow regulatory mechanisms due to exercise training.

Verification of exercise training must be established before conclusions can be made regarding the effects of training on intracoronary ACh's actions. Numerous studies have demonstrated a lower heart rate at rest and during submaximal exercise in endurance exercise studies, such as treadmill running or swimming. In agreement with those studies, the Exercise Trained swine showed significantly lower heart rates than the Sedentary swine at rest, during submaximal exercise (5 kph - 0% grade), and at the 5 min recovery period after a treadmill stress test. Both groups demonstrated a decrease in heart rate over the course of the study due to an expected decrease in heart rate with age as the heart enlarges (Barnard, 1975). This is indeed a factor to consider since the animals used in these studies were immature swine. In addition to the relative bradycardia of the Exercise Trained group, they also demonstrated a reduction in venous plasma lactate after completion of the treadmill stress test. These data support those studies that have observed a lower venous lactate at maximal exercise after training when compared to sedentary controls (McKirnan et al., 1986; Robinson and Harmon, 1941). The reduced lactate can be attributed to increased clearance (Donovan and Brooks, 1983), and/or decreased production of lactate (Hurley et al., 1984). Trained animals are believed to be more able to match lactate removal to lactate production than untrained animals. The mitochondrial oxidative pathway is enhanced with endurance exercise training due to the increase

in muscle mitochondria. Therefore, less lactate is produced, and it requires exercise at a higher percent of maximum oxygen consumption in the trained than in the untrained state to attain the same blood lactate concentraion (Hurley et al., 1984). The combination of lower heart rates and decreased circulating lactate after exercise, confirms that the training protocol utilized in these studies was successful in achieving a trained state.

Several studies have demonstrated an increase in atrial ACh with exercise training (De Schryver and Mertens-Strythagen, 1974; Herrlich et al., 1960). This increased concentration could be due to decreased enzymatic degradation of this vagal neurotransmitter by acetylcholinesterase. Measurement of resting arterial and venous enzyme levels demonstrated that exercise training had no significant effect on the resting levels of plasma acetylcholinesterase. These data are in agreement with studies by Herrlich et al. (1960) and Tipton et al. (1966) who also observed no change in cholinesterase levels after exercise training. With neurotransmitter breakdown unchanged, these data were interpreted as indicating an increase in cholinergic activity as being responsible for the decreased heart rate observed with training.

The effect of ACh on the coronary vasculature of the swine is vasoconstriction, and a consequent reduction in coronary blood flow. This effect has been documented in swine in both *in vitro* (Ito et al., 1979; Kalsner, 1985; Nakayama et al., 1988; Sakai, 1981), and *in vivo* (Cowan et al., 1987; McKenzie et al., 1988, Tondi et al., 1990) studies. Intracoronary bolus injections of ACh, in the dose range of 0.5

μg to 4.0 μg, were administered into the left anterior descending coronary artery. Subsequent reductions in coronary blood flow were noted, and % decrease in coronary blood flow calculated. At rest, there were no differences in the Sedentary and Exercise Trained group's responsiveness to these injections. Similar coronary blood flow reductions were demonstrated at each bolus injection in both groups (Figure 1).

Intracoronary bolus injections were also administered during a simulated bout of exercise. Norepinephrine was infused intravenously to achieve a 60 - 70 % increase in PRP (pressure-rate product). PRP has been documented as a reliable indicator of increased cardiac work (Coleman, 1971). ACh injections during this workload resulted in vasoconstriction of the coronary vasculature and reduction in coronary blood flow. There were no differences in responses to ACh in the Sedentary and Exercise Trained groups at any dose (Figure 2). An increase in cardiac work will dictate an increase in coronary blood flow, due to the increase in demand by the myocardium for a greater oxygen supply. Thus, the ACh-induced reductions in coronary blood flow during the NE infusion are lower than those observed at rest in both groups.

During recovery from an acute bout of exercise, sympathetic activity, which is stimulated during exercise, gradually decreases, whereas parasympathetic activity increases to regain resting predominance over control of heart rate (Robinson et al., 1966, Sadaniantz et al., 1988). Robinson et al. (1966) did a comparison of the control of heart rate in humans at rest and during exercise, and the efferent pathways

utilized, by studying the effects of autonomic blocking agents. Studies in healthy humans subjected to treadmill exercise have shown that if exercise was terminated abruptly, asystoles were noted during this "vagal recapture" period (Friedman and Friedman, 1990). A link was established between parasympathetic tone during recovery from exercise and post-exercise coronary artery spasm by Sadaniantz et al. (1988). In a case report of a patient with coronary artery disease, coronary artery spasm occurred soon after exercise when sympathetic stimulation is decreasing, and when parasympathetic tone is being reestablished. Sadaniantz et al. (1988) conclude that coronary artery spasm may mediate some of the cardiac events that occur immediately after exercise in individuals with minimal coronary artery disease. If exercise training does enhance parasympathetic cholinergic activity, then there may be an increased likelihood that the trained animals would be more sensitive to ACh-induced vasoconstriction with abrupt cessation of exercise. To determine the possibility of an altered sensitivity to ACh during this susceptible period, a 3.0 μg intracoronary bolus injection of ACh was administered 1 min after NE infusion was terminated. To make comparisons between groups more interpretable, μg ACh was converted to μM ACh concentration to take into account coronary blood flow differences at time of injection. Calculated μM ACh concentration in the Sedentary group was approximately 3-fold higher than the Exercise trained group, but the percent reduction in coronary blood flow was similar (Figure 5). These data support the view that during a state of parasympathetic neural dominance, endurance exercise

training enhances the responsiveness to muscarinic receptor-mediated vasoconstriction in the coronary vasculature.

To further assess the coronary vascular responsiveness to ACh, intracoronary infusions were administered to achieve steady reductions in coronary blood flow. Infusion of saline, the vehicle for ACh, served as control for the ACh infusions. There was no change in hemodynamic parameters due to this control infusion. A μg solution of ACh was infused to achieve steady state 10% and 30% reductions in coronary blood flow. Calculation of μM ACh infused demonstrated that approximately 0.4 μM ACh was required for a 10% reduction in coronary blood flow in both Sedentary and Exercise Trained groups. However, to achieve the same 30% reduction in coronary blood flow, the Sedentary group required about twice as much ACh as the Exercise Trained group. These data further support the belief that there is an increased sensitivity of the coronary vasculature to ACh induced coronary vasoconstriction due to endurance exercise training.

Prinzmetal's variant angina is believed to be caused by coronary arterial spasm (Prinzmetal et al., 1959; Prinzmetal et al., 1960), with enhancement of parasympathetic neural activity being a possible mechanism (Araki et al., 1983; Becker et al., 1987; Yasue et al., 1986). Coronary spasm has been induced in patients with variant angina by intracoronary injections of ACh, and suppressed by administration of the muscarinic receptor, competitive antagonist, atropine (Yasue et al.,1986), to confirm the involvement of the parasympathetic nervous system. With enhancement of the parasympathetic neural influence on cardiac function with

endurance exercise training, a greater coronary vasoconstrictive action would be expected with intracoronary ACh infusion.

Weiner et al. (1978) have demonstrated similar variant anginal symptoms during recovery after exercise testing. This response may be related to alterations in the autonomic balance during recovery after cessation of exercise. With the links established between the parasympathetic nervous system and coronary vasospasm at rest, and the parasympathetic nervous system and post-exercise coronary artery spasm, it seems very likely that training-induced modifications of muscarinic-mediated actions exist.

Intracoronary injection of atropine was administered to achieve muscarinic receptor blockade in the myocardium perfused by the LAD. There were no subsequent changes in hemodynamic or metabolic parameters following this injection. Although the parasympathetic nervous system is involved in the neural regulation of coronary blood flow, it does not appear to be involved in the resting tone of coronary vascular smooth muscle (Cowan and McKenzie, 1990). If endurance exercise training alters resting cholinergic activity of the coronary vasculature, one would expect changes in coronary blood flow after atropine administration. The Exercise Trained group demonstrated a higher post-atropine coronary blood flow than Sedentary, but it did not reach significance. As expected, coronary blood flow did not change significantly after muscarinic receptor blockade in the Sedentary group, in support of studies by Cowan and McKenzie (1990) supporting the lack of resting cholinergic tone in coronary vascular smooth muscle. Muscarinic receptor blockade was

successful in inhibiting the vasoconstrictive actions of ACh. The downward trend in heart rate observed after intracoronary atropine administration is possibly due to muscarinic receptor blockade in atrioventricular nodal tissue. The myocardium perfused by the LAD catheter may have included this nodal tissue in part or in whole, depending on each animal's LAD catheter location. Reductions in coronary blood flow with intracoronary bolus injections of ACh were prevented after atropine administration. These data verify that ACh-mediated vasoconstriction involves the muscarinic receptor of vascular smooth muscle.

Neural control of coronary blood flow involves activity of both the sympathetic and parasympathetic branches of the autonomic nervous system. A resting vasoconstrictor tone on the coronary vasculature has been documented (Mohrman and Feigl, 1978), and attributed to the sympathetic nervous system. This α-adrenergic receptor mediated vasoconstrictor tone is decreased with chronic exercise, providing an apparently greater role for β_2-adrenergic receptor-mediated vasodilation (DiCarlo et al., 1988; Gwirtz and Stone, 1984). Several studies have demonstrated altered sympathetic neural control of coronary vascular tone (Liang and Stone,1983; Oltman et al., 1990) in not only α-adrenergic, but also β-adrenergic mechanisms.

Due to the increased levels of catecholamines during exercise, a desensitization of β-adrenergic receptors is believed to occur (Butler et al., 1983; Friedman et al., 1987; Lefkowitz et al., 1984). This can be attributed to decreased coupling of the agonist to the receptor, or decreased coupling of receptor binding to a subsequent cellular response. Desensitization of β-adrenergic receptors is described

by Butler et al. (1983) as having a protective role in limiting the enhanced responsiveness that occurs initially with exercise. Hammond et al. (1988) showed that treadmill running resulted in substantial down-regulation of atrial β-adrenergic receptors, but an enhanced responsiveness of these receptors. Cousineau et al. (1977) demonstrated that physical training results in diminished sympathetic responses for a given absolute level of exercise. Conversely, Sigvardsson et al. (1977) demonstrated that β-adrenergic receptor sensitivity was not altered by physical training.

Norepinephrine was infused intravenously to achieve a 60 - 70% increase in pressure-rate product, and to assess differences in coronary vascular responsiveness to intracoronary bolus injections of ACh during increased cardiac work. If endurance exercise training does affect β-adrenergic responsiveness, then the concentration of NE required to simulate exercise at that workload would differ between Sedentary and Exercise Trained swine. A significantly greater amount of NE was required in the Exercise Trained group to achieve this workload. That the NE concentration was greater in the Exercise Trained group, lends support to the concept of desensitization of NE receptors with chronic exposure to catecholamines.

As expected with sympathetic stimulation via NE the following hemodynamic parameters were significantly increased from control values: MABP, PIP, PRP, dP/dt, and CBF. Exercise Trained myocardial oxygen consumption was significantly greater than Sedentary, due to greater contractility (dP/dt) and coronary blood flows. Increased myocardial oxygen consumption, induced by catecholamines, is primarily

due to augmentation of hemodynamic performance (Klocke et al., 1965; Sonnenblick et al., 1965), such as indicated by the increase in dP/dt. Down-regulation of β-adrenergic receptors has gained support as a cardiac modification that occurs with chronic exercise (Butler et al., 1982; Hammond et al., 1987; Savin et al., 1983). The enhanced cardiac performance in the Exercise Trained group, reflected in larger dP/dt, coronary blood flow, and MVO_2 values, supports that concept.

There is much controversy over altered catecholamine sensitivity with endurance exercise training. Dowell and Tipton (1970) administered isoproterenol and found a greater increase in heart rate in trained rats, indicating that training increased sinoatrial nodal sensitivity to catecholamines. Crews and Aldinger (1967) measured contractile force and demonstrated a decreased sensitivity to epinephrine with training. Östman et al. (1972) described decreased myocardial catecholamine turnover rate and less catecholamine excretion during exercise, in trained vs. non-trained rats. Östman and Sjöstrand (1970) demonstrated that training increased cardiac norepinephrine concentration in guinea pig hearts, whereas De Schryver et al. (1967, 1969) reported a decreased heart catecholamine concentration with training in rats. In this study, the circulating resting arterial level of norepinephrine was significantly greater in Exercise Trained than in Sedentary animals. The venous NE values for the Sedentary and Exercise Trained groups were similar, and reflects the combination of circulating and neural NE contributions, along with NE metabolism. This data points to either an increased norepinephrine neural component, and/or a decreased metabolism of NE in the Sedentary group.

In addition to a neural component, coronary blood flow is regulated by metabolic factors, such as lactate. Skeletal muscle adaptations to endurance exercise training include decreased lactate production due to increased mitochondrial oxidative pathways. This is reflected in lower circulating lactate concentration, as observed in venous plasma lactate measurements after treadmill stress test evaluations. The heart consumes lactate as a source of fuel for ATP production, as well as produces lactate during pathological anaerobic conditions. The heart's proficiency in utilizing lactate was assessed by calculating myocardial lactate consumption. Exercise Trained lactate consumption was significantly higher than the Sedentary group during simulated exercise with intravenous NE infusion. Coronary blood flow increased significantly during this period of increased oxygen demand in order to match supply with demand. These data support the expectation that the myocardium is able to consume lactate at a faster rate, and be more efficient in transporting oxygen with diminished anaerobic energy yield like skeletal muscle. Lactate consumption was also higher at rest in the Exercise Trained group than its Sedentary counterparts, but it was not significant. Increased myocardial efficiency in lactate metabolism during increased cardiac work was not observed during ACh induced reductions in coronary blood flow. During the intracoronary ACh infusions, lactate consumption fell significantly in both groups, when compared to their corresponding saline control infusions. This was due to a substantial decrease in extraction of lactate and reduced coronary blood flow. The Sedentary group appeared to be more metabolically compromised at the 30% reduction in flow, with

lactate consumption being significantly lower as compared to the 10% reduction in flow. The Exercise Trained group did not exhibit a significant change in lactate consumption at the 30 % coronary blood flow reduction. The Exercise Trained group did demonstrate higher lactate consumption than the Sedentary, but it was only significant at $p<0.08$. It appears that one way the coronary vasculature can combat an increased sensitivity to ACh-mediated vasoconstriction is to increase lactate consumption; whereas the Sedentary animals appear to be more metabolically compromised during the 30% reduction in coronary blood flow.

Hurley et al. (1984) suggested that decreased production of lactate in skeletal muscle could be a result of a greater proportion of pyruvate being channeled into the mitochondrial oxidative pathway and less into lactate. A cellular adaptation to chronic exercise is an increase in the size and number of mitochondria within the cell, causing an augmentation of oxidative phosphorylation (Holloszy and Booth, 1976). By increasing the mitochondrial capacity of the cell, there is a concomitant increase in the ability to oxidize pyruvate. If the decrease in venous lactate is due to this increased shuttling of pyruvate into the mitochondria for further oxidative metabolism, with more mitochondria available for this metabolic process to occur, a decrease in plasma pyruvate levels would be expected. With decreased pyruvate and similar lactate values, the venous lactate/pyruvate ratio should be elevated in the Exercise Trained group. The lactate/pyruvate ratio data support this reasoning. The Exercise Trained group had significantly higher lactate/pyruvate ratios than the Sedentary group at all metabolic parameter sampling times.

Physical regulators of coronary blood flow include myocardial wall tension and compression, and aortic blood pressure. Most studies of the effects of exercise training have noted small and insignificant changes in blood pressure (Åstrand and Rodahl, 1977; Martin et al., 1990), unless the subjects were initially hypertensive (Boyer and Kasch, 1970; Choquette and Ferguson, 1973). In this study, mean arterial blood pressure at rest was significantly lower in the Exercise Trained animals compared to their Sedentary controls; this evidence lends support to the theory that exercise training lowers MABP in normotensive subjects. Endurance exercise training is expected to increase tension per unit of cross-sectional area and rate of force development in the heart (Tibbits et al., 1978), which results in increased myocardial contractile performance. This enhancement of the inotropic state of the heart, i.e. increased contractility, is reflected in an increase in dP/dt (Barnard, 1975; Clausen, 1977; Schaible and Scheuer, 1985). Increased inotropicity is not always evident until the heart is stressed (Barnard, 1975). Comparison of dP/dt in the Sedentary and Exercise Trained groups revealed an increased dP/dt in the Exercise Trained animals both at rest and during a period of increased cardiac work (NE infusion). These data support the increased contractility response to endurance exercise training.

Controversy remains as to the underlying mechanisms responsible for the cardiovascular adaptations that result from endurance exercise training. Modifications of the regulatory mechanisms of coronary blood flow that may occur with chronic exercise involve the interaction of physical, metabolic, and neural

factors. The results of these studies support alterations in the physical regulatory component, reflected in decreased mean arterial blood pressure, and increased myocardial contractility at rest and during simulated exercise. Changes in the metabolic control of coronary blood flow were indicated in the enhancement of lactate consumption with training, indicating a possible decreased production and or increased metabolism of lactate. The interplay of the parasympathetic and sympathetic nervous system in the regulation of coronary blood flow remains controversial. Previous studies point to involvement of both, with more weight on the side of the parasympathetic component being responsible for the heart rate reductions observed with endurance exercise training. Parasympathetic influence, however, does not solely affect heart rate. Changes in the regulation of coronary blood flow with training may also occur. The responsiveness of the coronary vasculature to exogenous ACh in an animal model such as the pig, may provide important information about the involvement of the parasympathetic nervous system in vasoconstriction. Parasympathetic modifications that occur with endurance exercise training can then be elucidated. This study supports the enhancement of the pararsympathetic nervous system with endurance exercise training, as reflected in the increased responsiveness of the coronary vasculature to ACh after cessation of exercise and during a steady moderate reduction in coronary blood flow. This may indicate training-induced modifications in the number or affinity of muscarinic receptors, as well as an increase in the coupling of muscarinic receptor activation and its ultimate cellular response.

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