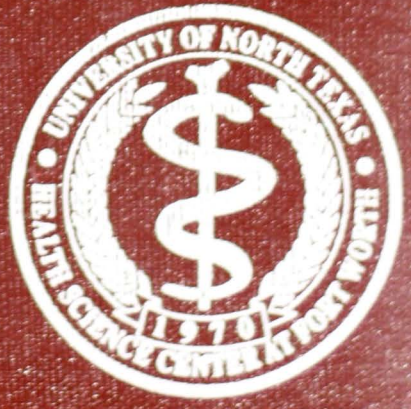
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Hawkins, Megan Nicole, The Effect of Fitness on Cardiac Work with and without Metoprolol. Doctor of Philosophy (Biomedical Science), July 2008; 128 pp; 3 tables; 17 figures; bibliography

Chronic endurance exercise training results in numerous morphological and functional adaptations of the cardiovascular and skeletal muscle systems. The mechanisms by which these adaptations occur, and their effect on the physiological response to exercise, have not been fully elucidated. In addition, the classic concept of the role of maximal oxygen consumption (VO_{2max}) as a parametric index of cardiorespiratory capacity has been questioned. Therefore the purpose of the investigations presented within this dissertation was to: i) retrospectively analyze 156 incremental exercise stress tests and supramaximal exercise tests to verify that VO_2 does indeed attain a maximal value; ii) evaluate the effects of cardioselective beta-adrenergic blockade on the ability to maintain cardiac work in average trained and endurance exercise trained subjects during moderate (45% VO_{2max}) and heavy (70% VO_{2max}) intensity cycling exercise; and iii) determine the effect of aerobic fitness on resting and peak leg vascular conductance and the change in central blood volume observed during the onset of cycling exercise. In the first investigation we demonstrated that highly trained runners capable of maintaining supramaximal workloads achieved a VO_2 that rarely exceeded the VO_{2max} value obtained during an incremental exercise stress test. In the second investigation we demonstrated that acute β_1-adrenergic receptor (βAR) inhibition reduced cardiac output, cardiac work and cardiac efficiency in endurance trained athletes during moderate and heavy intensity exercise. However, in average

trained individuals these same variables were not affected during moderate exercise intensity, but were reduced at heavy intensity exercise. We concluded that βAR blockade impaired the more efficient Frank-Starling mechanism in endurance trained athletes but remained functional in average trained subjects during moderate exercise intensities. In the third investigation we demonstrated that endurance athletes responded to the onset of exercise with a larger increase in central blood volume than average trained individuals. In addition, resting and post-ischemic leg blood flow and leg vascular conductance were greater in the exercise trained than in the average trained subjects. Therefore, endurance exercise training-induced adaptations of the skeletal muscle vasculature resulted in larger conductance capacity of the working muscle in response to increases in oxygen demand and enabled a greater increase in muscle blood flow from rest to exercise.

THE EFFECT OF FITNESS ON CARDIAC WORK DURING EXERCISE WITH AND WITHOUT METOPROLOL

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THE EFFECT OF FITNESS ON CARDIAC WORK DURING EXERCISE WITH AND WITHOUT METOPROLOL

DISSERTATION

Presented to the Graduate Council of the

University of North Texas Health Science Center at Fort Worth

In Partial Fulfillment of the Requirements

For the Degree of

DOCTOR OF PHILOSOPHY

By

Megan Hawkins, BS

Fort Worth, Texas

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Original Articles

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Hawkins MN, Quinton Barnes, Sushmita Purkayastha, Wendy Eubank, Shigehiko Ogoh, Peter B. Raven. The effects of aerobic fitness and beta-1 adrenergic receptor blockade on cardiac work during dynamic exercise. *Submitted to Journal of Applied Physiology, 2008.*

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The Reproducibility of Acetylene Re-Breathe Method for Determining Cardiac Output of Humans During Exercise. Poster Presentation. *Research Appreciation Day for the University of North Texas Health Science Center*, Fort Worth, Texas April 2, 2004.

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The Effects of Pulse Pressure on the Carotid Baroreflex During Mild Exercise. Poster Presenation by PB Raven. *American College of Sports Medicine National Meeting*, Nashville, Tennessee, June 2005.

Maximal oxygen uptake as a parametric measure of cardiopulmonary capacity. Poster Presenation. *Texas Chapter of American College of Sports Medicine Meeting*, Denton, Texas, February 24, 2006.

The Effects of Metoprolol on Total Cardiac Work During Submaximal Exercise. Poster Presentation. (2nd place) *Texas Chapter of American College of Sports Medicine Meeting*, Fort Worth, Texas, March 1, 2007.

Cardiac Work During Steady State Dynamic Exercise with and without Metoprolol. Poster Presentation. *Texas Chapter of American College of Sports Medicine Meeting*, Odessa, Texas, February 29, 2008.

Comparative Clinical Trial of a Novel Mini Chest Drainage System Versus a Standard Unit for Use in Thoracic Surgery: MERCURY STUDY. Poster Presentation by A. O'Yurvati. *Research Appreciation Day for the University of North Texas Health Science Center*, Fort Worth, Texas, March 28, 2008.

Cardiac Work During Steady State Dynamic Exercise with and without Metoprolol. Poster Presentation. *Research Appreciation Day for the University of North Texas Health Science Center*, Fort Worth, Texas, February 29, 2008.

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LIST OF ABBREVIATIONS

1/TI	thoracic admittance
ABP	arterial blood pressure
AT	average trained
A-VO_2diff	arteriovenous oxygen difference
βAR	beta-1 adrenergic receptor
CBV	central blood volume
CVP	central venous pressure
DBP	diastolic blood pressure
ET	endurance exercise trained
FAD	femoral artery diameter
FBV	femoral blood velocity
HR	heart rate
LBF	leg blood flow
LV	left ventricle
LVC	leg vascular conductance
LVM	left ventricle mass
MAP	mean arterial pressure
MRI	magnetic resonance imaging
mVO_2	myocardial oxygen consumption
PaO_2	systemic arterial oxygen tension

Qc	cardiac output
SBP	systolic blood pressure
SV	stroke volume
TI	thoracic impedance
TP	triple product
UT	untrained
VO_2	oxygen consumption

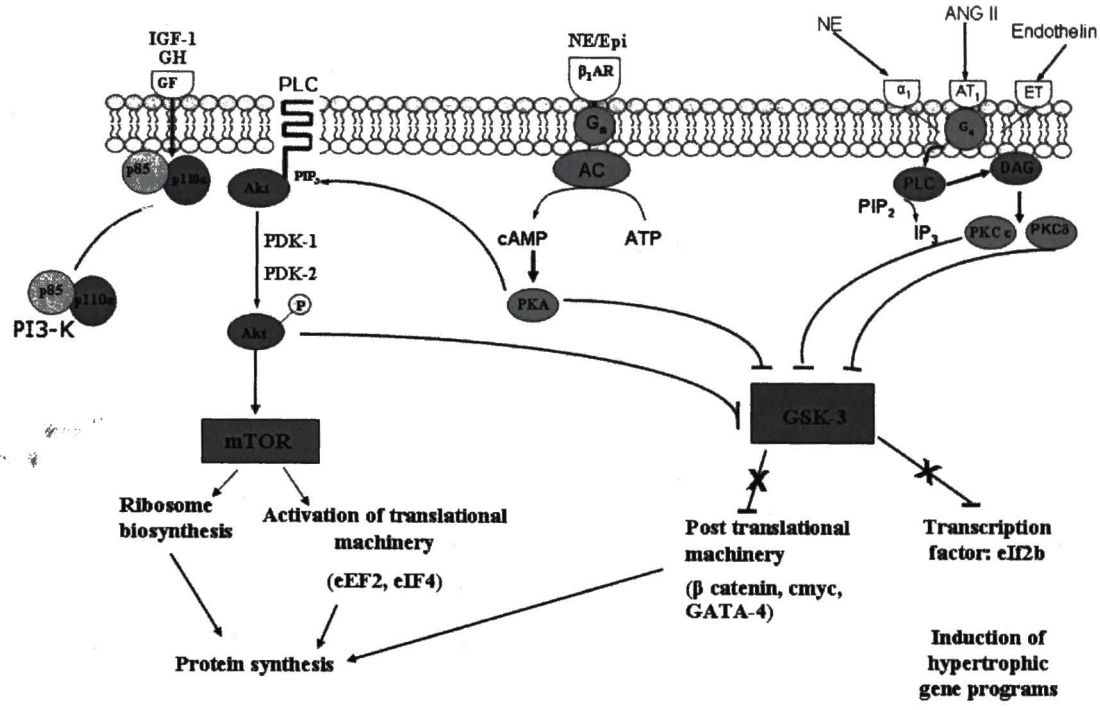
CHAPTER I

INTRODUCTION TO THE STUDY

The well-established concept of a parametric measurement of the circulation's capacity to deliver oxygen, i.e. maximal oxygen uptake (VO_{2max}), has recently been questioned (56-58). The questions raised focus on the concept of a plateau in oxygen uptake (VO_2) with increasing work rates developed by A.V. Hill in 1924 (37). Despite additional current investigations which have verified the presence of a plateau in VO_2 (18, 83) and the changes in hemodynamic variables confirming the circulatory capacity at VO_{2max} (61), there remains a competing concept of a "Central Governor." The central governor concept is argued as a protective mechanism emanating from the brain, to protect the heart against possible ischemic damage during supramaximal exercise (58). The need to provide more data regarding the plateau in VO_2 at supramaximal exercise requires individuals who are trained in endurance exercise and competition. Such data would adequately verify a limit to the amount of oxygen delivered by the circulation and its consumption by the tissues, despite further increases in exercise intensity. However, only the most elite athletes are mentally and physically capable of performing exercise at supramaximal workloads for a sufficient duration to adequately measure the oxygen uptake. In addition, the cardiac and vascular adaptations that occur with chronic endurance exercise training, i.e. increased cardiac (49, 88) and vascular

(12, 69) compliance and increased resting and exercise stroke volumes (80), suggest an increase in the heart's work efficiency while performing dynamic exercise.

In contrast, patients with ischemic heart disease are commonly prescribed a rehabilitative exercise training regimen in conjunction with cardio-selective $beta_1$-adrenergic receptor (βAR) antagonist therapy. The underlying principle of βAR antagonist therapy is to reduce sympathetic-mediated effects on heart rate and contractility, thereby attenuating increases in cardiac work during physical stress (86). The presence of the βAR blockade ensures that the myocardial metabolic demand remains below the threshold of ischemia-induced arrhythmia (62, 63). However, the potential βAR blockade-induced reductions in cardiac work may explain the lack of endurance exercise training changes in cardiac patients performing programs of cardiac rehabilitation (90); especially as there is a high density of βARs on the cardiomyocytes and there exists a βAR signaling pathway for physiologic cardiac hypertrophy (see figure 1).



Physiologic Cardiac Hypertrophy

Although this mechanism has not been identified in humans the chronic intermittent stimulation of these receptors during repetitive endurance exercise training bouts and recovery may play a role in the beneficial effects of endurance exercise training on the heart's structure and function. Preliminary investigations (unpublished data) in our laboratory sought to determine the effects of βAR antagonist therapy administered prior to each bout of chronic endurance exercise training performed five days per week for 12 weeks. Five pairs of age and VO_{2max} matched subjects completed the 12-weeks of training. One subject of each pair served as a control and performed the prescribed endurance training program without βAR antagonist therapy. The other subject of each pair performed the same training program in terms of intensity, frequency and duration with the added factor that two hours before beginning each training session the subject

ingested a non-selective βAR antagonist (timolol maleate). Prior to and following the endurance exercise training program the cardiac mass was determined using magnetic resonance imaging (MRI). We observed an increase in cardiac mass associated with the endurance exercise training of the control subjects that was twice that of the βAR blockade subjects. However, a major flaw of these preliminary findings was that βAR blockade is used clinically to reduce the HR and presumably the cardiac work of ischemic patients (62, 63, 86). Therefore, the reduced increases in cardiac mass of the healthy subjects training with βAR blockade may have been a result of a reduced amount of cardiac work related to lower exercise HR's compared to control subjects.

The large cardiac adaptations, i.e. hypertrophy and increased compliance, associated with endurance exercise training (5, 19, 23, 24, 87, 88) are minimal in ischemic heart disease patients (35) and, therefore, may not be the most predictive model of cardiac adaptations to endurance exercise training when combined with βAR blockade. Furthermore, in healthy subjects one would contend that the βAR blockade-induced bradycardia at rest and during exercise would result in an increase in stroke volume (SV) via the Frank-Starling mechanism of stretch-induced increases in force of contraction. Therefore, cardiac work would be maintained despite pharmacological reductions in contractility. Whether the increase in cardiac compliance and stroke volume of the endurance trained athlete can overcome the reduced contractility by means of a greater reliance on the Frank-Starling mechanism has not been evaluated. Indeed, a conclusive evaluation of cardiac work at rest and during submaximal exercise during βAR blockade

in healthy average trained (AT) or endurance exercise trained (ET) individuals has not been reported. This lack of information may be due to the difficulties associated with quantifying total cardiac work of exercising humans.

Preliminary investigations in our laboratory sought to determine the effects of a cardio-selective βAR antagonist (Metoprolol) during dynamic exercise in healthy individuals. In two AT subjects (VO_{2max} = 45-55 $mlsO_2$/kg/min) the cardiac work performed during dynamic exercise with βAR blockade for a given submaximal VO_2 was equal to or greater than the cardiac work during the same intensity exercise performed without βAR blockade. In contrast, the subject with below average fitness (VO_{2max} < 45 $mlsO_2$/kg/min), the cardiac work was less for a given intensity of dynamic exercise when performed with βAR blockade. These findings suggest that endurance exercise training, its resultant VO_{2max} and their relationships with exercise SV, cardiac compliance, maximal vascular conductance and muscle pump function (12, 49, 69, 80) appear to be important factors involved in maintaining cardiac work during βAR blockade.

It was the purpose of this dissertation to examine: i) whether VO_{2max} is a parametric measure of maximal circulatory capacity; ii) the cardiac work and cardiac efficiency in ET and AT subjects during dynamic exercise with and without βAR blockade; iii) the differences in peripheral vascular conductance in ET and UT individuals and its contribution to increases in venous return during dynamic exercise.

REVIEW OF RELATED LITERATURE

Total Body Maximal Oxygen Consumption:

Maximal oxygen consumption (VO_{2max}) was originally described by Hill and Lupton in 1923 (37) as the "oxygen intake during an exercise intensity at which actual oxygen intake reaches a maximum beyond which no increase in effort can raise it." Since this description VO_{2max} has been widely accepted as the measurement of maximal cardiorespiratory capacity to transport oxygen (18, 83). Objective, additional evidence that VO_{2max} was a parametric measure of cardiorespiratory capacity was established by Taylor, Buskirk, and Henschel in 1955 (83). In their investigation, 115 subjects performed maximal exercise tests on a motor-driven treadmill over a period of 3 to 5 visits. With each visit exercise intensity, determined by treadmill grade, was increased until VO_2 differed by less than 2.1 ml/kg/min on two subsequent visits (83) and was determined to be the subjects maximal VO_2 value. However, this concept has been recently questioned partly due to a lack of data validating the existence of a plateau when exercising at an intensity greater than that which elicits VO_{2max} (57, 58). The method of measuring VO_{2max} using the Taylor, Buskirk and Henschel protocol was not cost effective and lead to the development of a variety of progressive increases in exercise workload stress tests to establish a measure of VO_{2max} (4, 6, 53). A number of these modified tests did not always verify the presence of a plateau in VO_2 due to local muscle fatigue which caused the subjects to stop exercise at a VO_2 slightly less than their plateau value. Hence, an alternative proposal explaining the subjects' cessation of exercise was developed by Noakes (58). This proposal suggested a "central neural governor" which

was activated in response to afferent neural signals emanating from the myocardium which were presumed to identify impending ischemic injury to the heart. These neural signals were presumed to occur despite the lack of definitive changes in the electrocardiogram. It was proposed that maximal exercise elicited activation of the "central neural governor" which then triggered an efferent neural impulse in response to the physiological stresses of maximal exercise and would subsequently convey a signal to terminate exercise (58).

The lack of data obtained during exercise at supramaximal exercise intensities can be explained by the fact that only the most high-fit, proficient athletes are capable of exercising at supramaximal exercise work rates for a duration long enough to quantitatively confirm a plateau in VO_2. This limitation also suggests that endurance-trained high-fit athletes have acquired physiological adaptations that allow for the attainment and maintenance of VO_{2max}.

Exercise Training and Adaptations of the Cardiovascular System

It has been well documented in both humans and animals (8, 26, 75) that endurance exercise training results in numerous physiological adaptations. Endurance exercise training is responsible for augmentations in, but not limited to, the following: VO_{2max}; maximal cardiac output (Qc_{max}); resting, submaximal and maximal stroke volumes; maximal arteriovenous oxygen difference (A-V O_2diff); increases in resting blood and plasma volumes; and increased cardiac mass, cardiac compliance and ventricular chamber size (7, 8, 12, 14, 15, 22-24, 26, 28, 49, 75, 79, 80, 87, 88). In

addition, resting and submaximal exercise bradycardia and sinus arrhythmia are manifest after a program of endurance exercise training. These physiological adaptations to endurance training allow the cardiovascular system to work more efficiently in order to ensure that oxygen delivery meets the demands of the exercising muscles. The repeated work regime characteristic of exercise training for extended periods of time is responsible for the cascade of cellular and molecular events that leads to the training effect, and the physiological cardiac hypertrophy known as the "athletes heart (55)."

The cardiovascular adaptations developed in response to exercise training are time and intensity dependent and reliant upon the individual's genetic optimum. Therefore, elite endurance trained athletes commonly have a large heart weight to body weight ratio, as well as an increased VO_{2max}, SV_{max}, Q_{max}, and central blood volume (CBV) compared to an individual of average fitness as can be seen in figure 2 below.

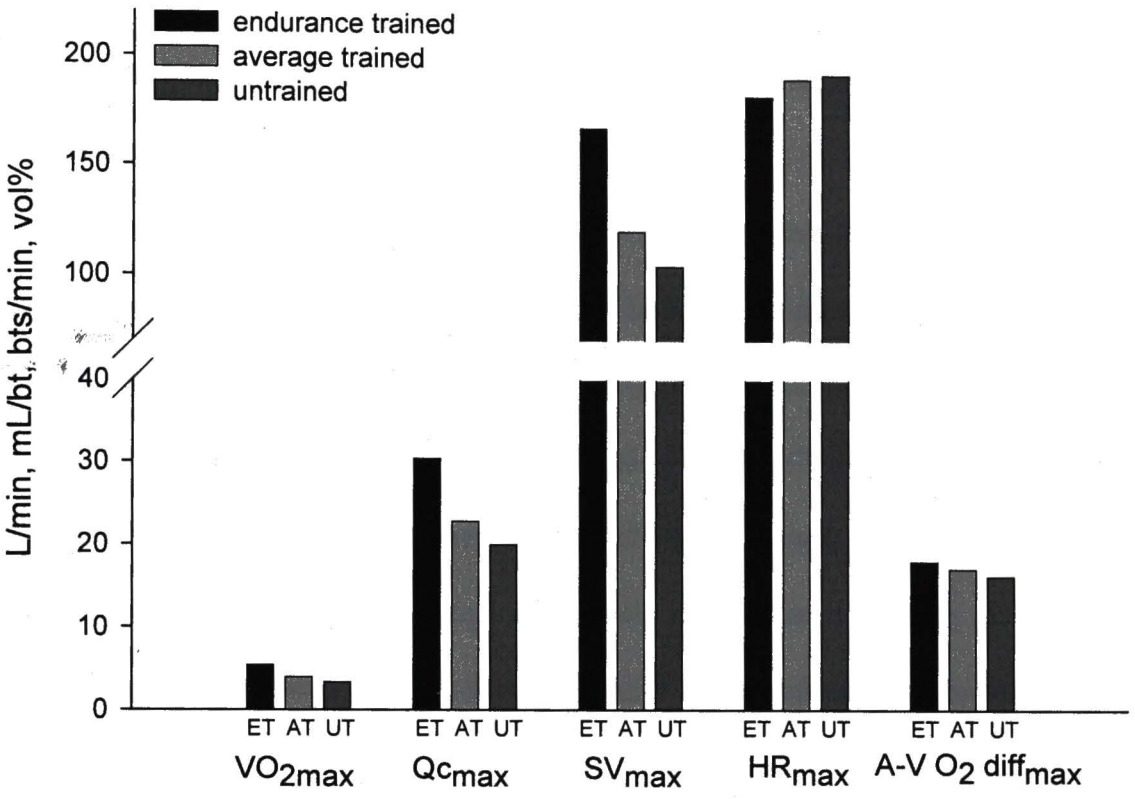


Figure 2. Histogram of physiologic variables reported in Olympic endurance trained (ET) athletes, average trained (AT) individuals, and untrained (UT) individuals. Data adapted from Blomqvist et al 1969 (9), and Saltin et al 1968(73).

The measure of VO_{2max} is commonly used as an indicator of cardiorespiratory capacity because in order for VO_{2max} to be increased Qc_{max}, SV_{max}, and/or A-VO_2diff_{max} must increase as well. Rowell et al. (72), and Saltin et al. (73) observed equal percentage increases in SV and A-VO_2 diff after 3 months of exercise training resulting in a 15%(72) and 33% (73) increase in VO_{2max}. However, further increases in VO_{2max} in previously fit subjects were attributed solely to increases in SV (20, 21, 73). The A-VO_2diff is increased with exercise training largely due to an increased diffusion gradient at the level of the tissues, thereby increasing oxygen uptake and decreasing venous PO_2 (9). The Qc

is a function of HR and SV. In addition, HR_{max} in adults is limited in its ability to increase beyond 190 to 200 beats/min. As the HR_{max} of sedentary, average fit and elite athletes are not different, the main contributor for the training-induced increase in Qc, and subsequently $VO_{2max,}$ is an increase in SV.

During a progressive incremental exercise stress test the SV of average-fit and untrained individuals plateaus at an exercise intensity of approximately 40% maximal oxygen uptake (VO_{2max}) (3, 28). Conversely, the endurance-trained athletes' SV's continue to increase to their individual maximal workload (3, 28, 32, 81). Gledhill et al. (28) demonstrated that the exercise-trained myocardium is more reliant upon enhancements in ventricular filling rather than ventricular emptying to increase SV. Diastolic filling time was reduced during exercise in trained subjects, however, diastolic filling volume was significantly larger (28). In addition, at near maximal exercise intensity the rate of ventricular filling is 86% greater than the rate of ventricular emptying in trained subjects (28). The SV is augmented in response to a training-induced increase in total circulating blood volume and increased end-diastolic filling volume. Chronic endurance training results in repeated volume loading of the heart. The effect is an increase in ventricular wall stress which is compensated for by increasing ventricular wall thickness and chamber diameter. Therefore, endurance exercise training results in the development of physiologic cardiac hypertrophy to reduce ventricular wall tension caused by repeated volume loads on the heart.

Endurance exercise training and physiologic cardiac hypertrophy

In high fit individuals, long duration exercise sessions result in increased sympathetic nerve activity and increased circulating blood volume that lasts well into recovery (2, 15, 16, 27, 30, 74). Greater circulating blood volume intensifies ventricular wall stress similar to that seen in pathological volume overload, and a resultant concentric and eccentric remodeling of the cardiomyocytes occurs (33). Due to the large reserve capacity of the veins, increases in venous tone and the muscle pump result in increased venous return and ultimately CBV at the onset of exercise (29, 52, 76, 91). During each recovery phase from an acute exercise bout there is a significant expansion of plasma volume (78), which progressively accumulates with multiple bouts of a chronic exercise training program resulting in chronic plasma volume expansion. Increased wall stress as a result of exercise-induced volume loading or pathologically-mediated volume overload signals cardiomyocyte remodeling in order to reduce ventricular wall tension (31). The Law of Laplace for a thin walled sphere states: wall stress = (pressure x radius)/(2 x wall thickness). Therefore, a thicker ventricle balances chronic pressure or volume overloads characteristic of physiological and pathological cardiac hypertrophy (65). In hypertension-induced pathological cardiac hypertrophy wall thickness is increased to reduce wall tension from chronic pressure loads. However, coronary blood supply is not altered and the inner myocytes are deprived of an adequate oxygen supply. Eventually, apoptosis of the ventricular myocytes occurs causing a reduction in wall thickness, decreased contractility, repeated volume loading of the heart, and enlargement of ventricular chamber diameter (85). In contrast, endurance exercise training increases

angiogenic signaling cascades and remodeling of the coronary vasculature ensuring adequate oxygen delivery to the larger ventricle (48). Exercise training that entails extended periods of volume loading results in greater increases in left ventricular internal diameter and wall thickness. Pelliccia et al. (66) reported bicyclists with an average left ventricle internal diameter of 55 mm and a wall thickness of 10 mm whereas the average left ventricular internal diameter of a high board diver was 50 mm and wall thickness was 8.7 mm. Competitive rowers have been reported as having some of the largest heart weight to body weight ratios with internal dimensions and wall thicknesses of 56 and 11 mm respectively and reported maximum values of 59 mm and 14 mm (13).

Iwasaki et al. (41) trained previously sedentary subjects for one year to participate in a marathon, triathlon, or cycling race. They determined that the observed concentric hypertrophy developed with endurance exercise training takes approximately 3 months to develop to a significant degree, and the enlargement of left ventricle chamber diameter evolves significantly within 6 months (41). Therefore, in order to determine the mechanisms with which physiological hypertrophy occurs in humans, endurance training programs must be performed for at least 6 months. In addition, exercise intensities which become progressively more difficult from 60% VO_{2max} to 85% VO_{2max} ensure chronic increases in ventricular wall stress and initiation of the development of cardiac hypertrophy.

Effect of Fitness on Skeletal Muscle Vasculature

In addition to the physiological hypertrophy that develops in the trained myocardium, there is hypertrophy of the skeletal muscle and angiogenesis of the skeletal muscle vascular bed in response to exercise training (39, 45). Endurance exercise training augments the conductance capabilities and the microcirculation of the leg skeletal muscle vasculature. Venous cuff occlusion combined with calf exercise produced a 29-fold increase in leg blood flow in endurance exercise-trained subjects compared to a 19-fold rise in sedentary subjects, although baseline flows were no different (79). Furthermore, one-legged exercise training produced a significant increase in leg blood flow during one-legged exercise compared to pre-training (45). The increased blood flow was accompanied by a 20% increase in capillarization of the trained muscle vasculature (45). These data suggest that the exercise-trained skeletal muscle vascular bed has a greater blood flow capacity than that of untrained individuals.

Exercise generates skeletal muscle contractions which compress the veins, termed "the muscle pump" and rapidly increases venous outflow from the muscle and venous return to the heart (77). It has also been demonstrated that endurance exercise-trained individuals have a larger circulating blood volume than those of average individuals (15). It is possible that exercise training enhances the effectiveness of the muscle pump due to augmentations in skeletal muscle mass, blood flow capacity and circulating blood volume. Moreover, the muscle pump has been proposed to further enhance muscle blood flow by lowering venous pressure post-contraction and enlarging the pressure gradient across the muscles' vascular bed resulting in greater arterial inflow (25, 67). If muscle

pump function is enhanced in endurance-trained individuals it may also assist in further increasing arterial blood flow to the leg vasculature during dynamic exercise.

The main function of the muscle pump is to maintain cardiac filling pressure (71). In heart transplant patients dynamic exercise elicited a continuous increase in central venous pressure (CVP) (59). In contrast, the CVP of healthy, normal subjects increased immediately and then reached a plateau within 3 minutes of the onset of exercise (59). Cardiac output increased in both groups of subjects (59). These data indicate that with cardiac denervation, muscle contractions play a greater role in augmenting venous return and that the cardiac output is largely increased via the Frank-Starling mechanism rather than sympathetically mediated increases in contractility.

Effects of Fitness on Myocardial Compliance

As we have established, endurance exercise training results in physiologic cardiac hypertrophy, skeletal muscle hypertrophy, angiogenesis of the skeletal muscle vasculature and an increased circulating blood volume. In relation to these adaptations, the myocardium of endurance athletes becomes more compliant (49). For a given volume load at rest, the trained heart of ET athletes operates on a steeper portion of the Frank-Starling curve. Therefore, for a given change in filling pressure, due to volume loading, ET athletes had a greater capacity to increase SV (49). In addition, other investigations have demonstrated that the athletes enhanced ventricular filling is a result

of greater use of the Frank-Starling mechanism which is more energy efficient and can further increase SV at high exercise intensities (17).

Physiologic cardiac hypertrophy increases cardiac work efficiency and attenuates myocardial perfusion compared to untrained myocardium operating at the same given external exercise workload (46). However, at an exercise intensity performed at the same myocardial workload there was a non-significant trend towards an attenuated increase in myocardial perfusion in exercise-trained compared to average-fit subjects (46). Therefore, the question as to whether physiologic cardiac hypertrophy elicits a more efficiently functioning myocardium for the same cardiac workload warrants further investigation.

Indices of myocardial oxygen consumption

Measurement of myocardial oxygen consumption (mVO_2) involves catheterization of the coronary sinus and use of an indicator dye, and is therefore difficult to directly measure in humans during exercise. Indirect correlates of mVO_2 include heart rate, rate-pressure product, tension-time index, the triple product, pressure work index (PWI), pressure-volume area under the curve (PVA) and total energy requirement (10, 42, 44, 54, 70, 82). An adequate index of mVO_2 is a necessity in an experiment designed to explore whether an increase in submaximal SV during βAR blockade maintains external work of the heart due to a greater force of contraction via the Frank-Starling mechanism. The measurement of triple product (TP) accounts for augmentation of SV during changes in ionotropic state, such as βAR blockade. The equation for calculating

the triple product is as follows: cardiac work ≈ HR x SBP x stroke work. Rooke and Feigl (70) invasively measured mVO_2 with an experimental preparation used to control stroke volume via arteriovenous shunts and blood pressure via a pressure control reservoir and phenylephrine infusion in closed-chest anesthetized dogs. They determined TP was well correlated with mVO_2 during changes in stroke volume r=0.926, and during ionotropic changes, r=0.833 (70).

SPECIFIC AIMS

The validation of the measurement of maximal oxygen uptake (VO_{2max}) has singular importance to the field of exercise physiology. Therefore, we undertook a separate retrospective study of highly trained endurance athletes to validate the measurement of VO_{2max}. Subjects performed an incremental treadmill VO_{2max} test via a modified Astrand protocol and a subsequent supramaximal exercise test. The specifics of these tests, the analysis of the data, and the finding were published in Medicine and Science in Sports and Exercise, (2007)39, pp 103-107.

Pathological cardiac hypertrophy has been documented as a phenotypical adaptation to cardiovascular diseases such as hypertension and heart failure. Patients diagnosed with hypertension are prone to concentric remodeling and thickening of the left ventricle, whereas heart failure patients develop eccentric myocardial hypertrophy resulting in dilatation of the left ventricle chamber. While the pathological induction of cardiac hypertrophy is unfavorable, endurance trained athletes develop exercise induced physiological cardiac hypertrophy in which thickening of the ventricles and enlargement

of the ventricular chambers allow the heart to function in a manner that allows for more efficient work per beat. While it has been well established that these cardiac adaptations occur, the exact mechanism(s) responsible for these changes remain unclear.

Previous animal studies (34, 50, 60) suggest that chronic infusion of catecholamine results in a significantly increased left ventricular mass which was attenuated by co-administration of a non-selective β-adrenergic receptor blocking drug. In addition, increased ventricular mass observed in swim-trained rats was attenuated with chronic βAR blockade. These findings suggest the βAR signaling cascade is involved in the development of cardiac hypertrophy. Complications in examining the role of βARs in physiological hypertrophy arise when considering the decreases in external cardiac work thought to occur with administration of βAR antagonists due to pharmacologic decreases in heart rate and contractility. Therefore, it is possible that the decreased mechanical work of the heart is responsible for the attenuation in physiological hypertrophy observed in the aforementioned animal studies, not βAR inhibition per se. However, we propose that at high exercise intensities βAR blockade-induced bradycardia will increase ventricular filling time therefore, increasing cardiac filling volume. Larger filling volumes will stretch the ventricles' sarcomeres to a more optimal length, allowing for a more forceful ventricular contraction via the Frank-Starling mechanism. Therefore, SV will increase and external cardiac work will be maintained despite βAR inhibition related attenuations in contractility. Preliminary data obtained in our laboratory have indicated that individuals with a consistent exercise routine (VO_{2max} 45-55 $mlO_2/kg/min$) have an equal cardiac work during high intensity exercise with and without βAR blockade. In

contrast, sedentary individuals (VO_{2max} < 40 mlO_2/kg/min) appear to have a decreased cardiac work during all intensities of exercise with βAR blockade.

In addition, physiologic cardiac hypertrophy has been implicated in increasing the efficiency of cardiac work due to a decrease in myocardial perfusion for a given cardiac external workload when compared to healthy untrained subjects (46). When exercising at the same percent VO_{2max}, however, myocardial perfusion was not significantly different but there was a trend toward a decrease in myocardial perfusion in endurance trained subjects compared to healthy untrained subjects.

Therefore, we hypothesize that mVO_2 is not attenuated by cardioselective sympathetic inhibition in endurance exercise trained athletes due to increased skeletal muscle vascular conductance during exercise in conjunction with larger cardiac filling volumes. We further hypothesize that endurance exercise training-induced cardiac remodeling and the resultant larger cardiac mass results in an increased cardiac efficiency.

We will test these hypotheses by examining the following specific aims:

I. **To test the hypothesis** that myocardial oxygen consumption is not affected by βAR blockade in endurance trained athletes but is affected in average trained individuals during submaximal exercise.

II. **To test the hypothesis** that endurance exercise training increases the efficiency of cardiac work for a given external exercise workload.

III. **To test the hypothesis** that a larger maximal peripheral vascular conductance in endurance trained athletes allows for an increase in CBV during submaximal cycling exercise compared to average trained individuals.

These specific aims have been formulated to determine a cross-sectional difference between cardiac work, cardiac efficiency and peripheral vascular conductance in endurance trained athletes compared to average trained individuals. One set of experiments was designed to test specific aims I, II and III. In addition, an initial experiment was designed to ensure that a maximal exercise stress test for the measurement of VO_{2max} is a parametric measure of maximal cardiorespiratory capacity. These experiments are explained in detail in chapters 2, 3 and 4. However, a general description of the experimental design, experimental protocols and methods used to address the goals of the investigation is provided below.

EXPERIMENTAL DESIGN

Maximal Oxygen Uptake as a Parametric Measure of Cardiorespiratory Capacity

Maximal oxygen consumption (VO_{2max}), and VO_2 at supramaximal exercise intensities were measured on two separate experimental days. Highly competitive

collegiate runners were recruited in order to ensure supramaximal exercise was accomplished for a duration long enough to obtain an accurate maximal VO_2 measurement. Incremental graded exercise on a treadmill via a modified Astrand protocol (4) was used to determine VO_{2max}. The VO_2 was measured using the Douglas bag method; gas fractions were analyzed by mass spectrometer (Marquette MGA 1100); and ventilatory volume was measured with a Tissot spirometer or dry-gas meter (Collins). The following day a supramaximal exercise test was performed at a workload that required a VO_2 approximately 30% greater than that achieved during the maximal stress test. The peak VO_2 measurements obtained during each test were then compared by paired *t*-tests. These results validated that VO_{2max} is an invariable parameter that defines an individual's cardiorespiratory systems ability to transport oxygen to the various tissues of the body (18, 36, 83).

The effects of aerobic fitness and beta-1 adrenergic receptor blockade on cardiac work during dynamic exercise

Cardiac work was measured during exercise with and without metoprolol in average trained (AT, VO_{2max} 40-50 mlO_2/kg/min) and endurance trained athletes (ET, VO_{2max} > 60 mlO_2/kg/min) before and after administration of metoprolol, a selective β_1-adrenergic receptor (βAR) antagonist. On the initial visit to the laboratory subjects

performed an incremental maximal exercise stress test on a stationary cycle ergometer (SciFit) to volitional exhaustion. The VO_{2max} value obtained served as an index of aerobic fitness and was used to determine the exercise workload performed during the following visit. In all subjects, cardiac echocardiography was performed via Doppler ultrasound (Phillips HDI 5000) to enable measurement of left ventricle (LV) wall thickness, chamber diameter, and mass in both groups of subjects. On the second visit each subject performed dynamic steady-state exercise at 45% and 70% VO_{2max}. Cardiac output (Qc), systolic blood pressure (SBP), diastolic blood pressure (DBP), heart rate (HR), and VO_2 were continuously monitored and recorded during steady-state exercise. The aforementioned variables were used to calculate cardiac work and cardiac efficiency during submaximal exercise with and without metoprolol in AT and ET subjects.

Effects of aerobic fitness on peripheral vascular conductance and central blood volume during submaximal exercise

Peak leg vascular conductance (LVC) post-ischemia was measured in the same ET and AT subjects mentioned above. Doppler ultrasound (Phillips HDI 5000) technology was used to determine resting and peak LVC at the common femoral artery post-venous cuff occlusion in conjunction with calf-raising exercise. Additionally, thoracic impedance (TI) was measured from rest to low intensity (50 W) dynamic cycling

exercise. Changes in thoracic admittance (1/TI) are highly correlated with measurements of central blood volume (CBV) (11). Therefore, changes in CBV (ΔCBV) from rest to exercise onset were observed and served to determine whether activation of the skeletal muscle pump with exercise onset increases CBV to a larger extent in ET compared to AT subjects.

METHODS

A brief description of the methodology used to determine VO_{2max}, cardiac work, cardiac efficiency, left ventricular mass, peripheral vascular conductance, and thoracic impedance as determined by these investigations, is provided in this section of the chapter.

Graded Treadmill Evaluation and Supramaximal Test

Incremental exercise on a treadmill via a modified Astrand-Saltin protocol (4) was used to determine VO_{2max} in distance-trained runners. The incremental test was performed on a calibrated treadmill during which men ran at 9.0 mph and women ran at 8.0 mph at 0% grade for 2 min. The grade was increased 2% every 2 min until volitional exhaustion was achieved. Exhaustion occurred approximately 10-12 minutes after the initial increase in grade. The VO_{2max} was defined as the highest VO_2 measured from the

last 40 sec Douglas bag collection. On a separate day subjects completed a supramaximal treadmill run to confirm the VO_{2max} value obtained in the incremental test. In addition, anaerobic capacity was estimated from the accumulated oxygen deficit, according to the method of Medbo et al (51). Supramaximal exercise began with subjects running at 8% grade with the speed chosen individually to exhaust the subject between 2 and 4 min. VO_{2max} was defined as the highest VO_2 obtained if a minimum of three 45 sec Douglas bags were collected.

Stationary Cycle Ergometer Maximal Exercise Stress Test

The subjects in specific aims I, II and III performed a graded exercise stress test on a stationary electrically braked cycle ergometer (Scifit) to volitional exhaustion in order to determine VO_{2max}. The initial workload of the exercise stress test was 50 watts and each minute the workload was progressively increased dependent upon the individual subject's predicted aerobic fitness to ensure that volitional exhaustion was reached within 6-10 minutes (4). The AT subjects pedaled at 60 rpm and the ET subjects were allowed to pedal at the frequency at or above 60 rpm that they used during competition.

Cardiac Output, Cardiac Work and Cardiac Efficiency

Cardiac output was measured via the acetylene rebreathe technique as described previously (84). The subjects inspired a breath from a tank containing a gas mixture of 0.5% acetylene, 9% helium, 36% oxygen, and 54.5% nitrogen. The subjects then rebreathed the gas mixture into a 5L bag for 4-6 breaths. A mass spectrometer (Perkin-

Elmer MGA-1100A) continuously monitored the progressive decrease in the concentration of acetylene in the bag and a customized computer program calculated the rate of disappearance. The customized computer program was programmed with the diffusion rate and solubility of acetylene in human blood and was used to calculate Qc. The SV was calculated as Qc divided by HR. Unpublished data from our laboratory verified the reproducibility of the acetylene rebreathe technique. Ten subjects, recruited separately from the subjects of specific aims I, II and III, performed cycling exercise at two separate exercise intensities (40% and 60% VO_{2max}) on two separate visits to the laboratory. The order in which the exercise intensity was performed was randomized for each visit with a 2 hour break between each session to allow hemodynamic variables to return to baseline. The Qc was measured every 5 minutes at rest and during exercise. We found the technique to produce highly reproducible Qc values (r^2= 0.88) for the two visits. In addition, the average coefficient of variation for the low and high intensity workload was 7.2% and 8.4%, respectively.

Respiratory gases were measured through a mouthpiece attached to a saliva trap and a turbine volume transducer to determine breath-by-breath measurements of metabolism. Noseclips were utilized so that respired oxygen (O_2), carbon dioxide (CO_2) and partial pressures (PO_2 & PCO_2) could be measured from a sampling port in the saliva trap via mass spectroscopy. Expired and inspired air flows were measured using a turbine transducer which transmits a voltage output to the ventilation measurement module (VMM; Alpha Technologies, Inc). Signals were converted from analog to digital using a laboratory minicomputer (Dell Inc., 466/T) for on-line, breath-by-breath computation of

VO_2, carbon dioxide output (VCO_2), respiratory exchange ratio (RER), and minute ventilation (VE) using customized computer software. The data were displayed on a graphics terminal during exercise as 15 second averages.

Cardiac work was calculated via the triple product (TP) of HR, SBP, and SV recorded simultaneously at rest and during cycling exercise. Rooke and Feigl (70) compared TP to invasive mVO_2 measurement in the same preparation mentioned above. They determined TP was well correlated with mVO_2 during changes in stroke volume r=0.926, and during ionotropic changes, r=0.833 (70). Cardiac efficiency was defined as the amount of work performed per amount of maximal work possible (64). For the purposes of the current investigation we have defined myocardial efficiency as the amount of cardiac work performed for a given total body oxygen uptake. A comparison of cardiac work and cardiac efficiency between ET and AT individuals with and without metoprolol were calculated and analyzed via two-way analysis of variance.

Echocardiography

Echocardiographic measurements of LV mass, wall thickness and chamber diameter were obtained via Doppler Ultrasound Echocardiography (Phillips HDI 5000) using 2-4 MHz transducer interfaced with a videographic recorder (Sony SVO-1410). All echocardiographic measurements were made by the same qualified individual. The transducer was placed between the second and fourth intercostal space lateral to the sternum. Measurements were recorded in parasternal long axis and short axis views. The transducer was angulated to ensure simultaneous visualization of the left ventricular

posterior wall and septum at the chordal level between the free edges of the mitral leaflets and the tips of the papillary muscles. The following left ventricular dimensions were obtained using M-mode echocardiography in parasternal long axis view at end-diastole (47): 1) intraventricular septal thickness; 2) left ventricle internal diameter and 3) posterior wall thickness. Left ventricular mass (LVM) (g) was determined using the Penn convention (46):

LVM = 1.04 x [(end diastolic diameter + PWT + IVS thickness)^3 – end diastolic diameter^3] – 13.6g.

Leg Vascular Conductance

Leg blood flow (LBF) and leg vascular conductance (LVC) were measured using Doppler ultrasound technology (Phillips HDI 5000). In order to determine LBF, we placed a 4-7 MHz duplex pulsed transducer probe on the skin over the common femoral artery, 2 to 3 cm proximal to the bifurcation of the common femoral artery into the profundus and superficial femoral artery (1, 38, 43, 89). This location was chosen to limit the amount of noise and artifact resulting from turbulence originating from the bifurcation. Femoral artery diameter (FAD) and femoral blood velocity (FBV) were measured simultaneously in pulsed format with live images of both 2D vessel image and M-mode velocities tracing present on the same screen. Therefore, continuous measures of LBV and vessel diameter allows for the determination of LBF on a beat-to-beat basis for a given unit of time (i.e. cardiac cycle). Mean LBF was calculated using the formula:

LBF = LBV * π * radius^2. Radegran et al (68) compared flow measurements with Doppler ultrasound technology and thermodilution during different one-legged knee extension exercise intensities and demonstrated a high relationship between the two measures of flow (r=0.974). The LVC was be determined using the following equation: **LVC = femoral blood flow/mean arterial pressure**. Peak LVC was defined as the average of the first 10 beats of the plateau values (4 seconds after release of venous occlusion cuff) and the corresponding MAP. Beat-to-beat arterial blood pressure was measured non-invasively by a servo-controlled finger photoplethesmogram (Finometer, Finapres Medical Systems) placed on the middle finger of the left hand. Using this method, changes in mean arterial pressure (MAP) were recorded at rest and post-cuff occlusion, and have previously been demonstrated as no different from direct ABP measurements at rest and during dynamic exercise (40).

Impedance Cardiography

Impedance cardiography (Minnesota Impedance Cardiograph, Model 304B) was used to measure thoracic electrical impedance (TI). Changes in thoracic admittance (Δ1/TI) between a high and low frequency reflect the distribution of erythrocytes in the body, and therefore, can be used as an index of beat-to-beat ΔCBV as previously described (11). Impedance tape is placed around the subjects' upper and lower neck approximately two inches apart. Impedance tape is also placed below the sternum and

above the navel separated by approximately 5 inches. The Δ1/TI was measured while sitting on the stationary bike at rest and continued through the onset of exercise at 50 W in both ET and AT subjects. The Δ1/TI was calculated for a 30 sec period at rest, and during exercise at 50W and the values were compared for both groups of subjects to determine the difference in ΔCBV with exercise onset between ET and AT individuals.

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CHAPTER II

MAXIMAL OXYGEN UPTAKE AS A PARAMETRIC MEASURE OF CARDIORESPIRATORY CAPACITY

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ABSTRACT

Maximal oxygen uptake (VO_{2max}) was defined by Hill and Lupton in 1923 as the oxygen uptake attained during maximal exercise intensity which could not be increased despite further increases in exercise workload, thereby defining the limits of the cardiorespiratory system. This concept has recently been disputed due to the lack of published data reporting an unequivocal "plateau" in VO_2 during incremental exercise. The purpose of this investigation was to analyze a large number (N=156) of incremental exercise tests in competitive middle distance runners and their subsequent supramaximal exercise tests to determine conclusively if VO_2 does indeed attain a maximal value which subsequently plateaus or decreases with further increases in exercise intensity. The 52 subjects (36 men, 16 women) performed incremental exercise tests, while measuring VO_2 using the Douglas bag method. On the following day the subjects returned for a supramaximal test during which they ran at 8% grade with the speed chosen individually to exhaust the subject between 2 and 4 min;VO_2 at supramaximal exercise intensities (30% above incremental VO_{2max}) was measured continuously. The VO_{2max} measured during the incremental test (63.3 $\pm$ 6.3 ml/kg/min; mean $\pm$ SD) was indistinguishable from the VO_{2max} during the supramaximal test (62.9 $\pm$ 6.2, N=156; $p = 0.77$) despite a sufficient duration of exercise to demonstrate a plateau in VO_2 during continuous supramaximal exercise. These data provide unequivocal evidence that there is indeed a peak and subsequent plateau in VO_2 during maximal exercise intensity. Therefore,

VO_{2max} is a valid index measuring the limits of the cardiorespiratory systems' ability to transport oxygen from the air to the tissues at a given level of physical conditioning and oxygen availability.

INDEX TERMS: treadmill testing, running, Douglas Bag

INTRODUCTION

Maximal oxygen uptake (VO_{2max}) was first described by Hill and Lupton in 1923 as "the oxygen intake during an exercise intensity at which actual oxygen intake reaches a maximum beyond which no increase in effort can raise it (6)". They further postulated that the measurement of VO_{2max} defined the limits of the cardiovascular and respiratory systems ability to transport oxygen.

Subsequently, the concept that the measurement of a plateau of VO_{2max} is a quantifiable and reproducible parameter of the cardiorespiratory system's ability to maximally deliver oxygen has been repeated sufficiently that it has achieved near universal acceptance. However, the concept of a truly maximal oxygen uptake and the resultant plateau with supramaximal exercise, as well as the experimental design of A.V Hill's original investigation have been challenged (14,15). The principal argument in this challenge is that Hill and Lupton did not "prove" that VO_{2max} and the subsequent plateau were ever achieved because they did not attempt to validate the existence of a plateau by running at speeds higher than the maximal speed at which VO_{2max} was measured.

The logic involved in developing the challenge to the concept of VO_{2max} has been rebutted by Bassett and Howley (1,2). Howley (8) has correctly identified that the modern equipment and data acquisition systems used today generally impose a measurement rigor, in terms of breath-by-breath data collection and the identification of

VO_{2max}, that has in many investigations resulted in an inability to identify a clear plateau of VO_2 in standard incremental tests. However, many of the contrary arguments have not utilized data from experiments that employed repeated measurements of VO_{2max} using the same methodologies applied by Taylor, Buskirk, and Henschel in which short duration supramaximal speeds were used (19). Moreover, it may be difficult for all but the most accomplished athletes to remain running long enough at supramaximal treadmill work rates to confirm that VO_2 has truly plateaued.

Recently, Day et al. (3) demonstrated that while a plateau in VO_2 may not be observed during every incremental exercise test, the VO_{2max} obtained is useful in determining cardiorespiratory capacity during exercise. This conclusion was based upon the linear correlation between incremental VO_{2max} and a constant load VO_{2max}. However, only a small number (N=6) of subjects in this experiment actually underwent a supramaximal test to confirm that VO_{2max} had been achieved, and even in these subjects, the increase in work rate above incremental max was small, and within the measurement error of oxygen uptake; it is still necessary to provide conclusive evidence that the VO_{2max} obtained during incremental exercise is representative of the actual limitations of the cardiorespiratory system. Therefore, it was the purpose of this investigation to analyze 156 incremental VO_{2max} tests, performed in highly competitive collegiate middle distance runners, and their subsequent supramaximal exercise tests to verify that VO_2 does indeed attain a maximal value at which, despite an increase in work intensity, there is no further increase in the measurement of VO_2.

METHODS

Subjects

Fifty-two well-trained distance runners (35 M, 16F), who were participating in an altitude training study completed three series of VO_{2max} tests: *i*) at the end of a two-week lead-in training phase; *ii*) after 4 weeks of sea-level training; and *iii*) after another 4 weeks of training while living at high altitude or sea-level and training at high altitude or sea-level. Subject and experimental details have previously been reported (9,10). All subjects gave their voluntary written informed consent to a protocol approved by the Institutional Review Board of the University of Texas Southwestern Medical Center at Dallas.

Treadmill evaluation.

VO_{2max} was measured with a modified Astrand-Saltin protocol (10) involving incremental exercise on a treadmill. After a 15-min warm-up, subjects ran at 9.0 miles/h (mph) for men and 8.0 mph for women at 0% grade for 2 min. The grade was then increased 2% every 2 min until volitional exhaustion, which usually occurred 10–12 minutes after the initial increase in grade. Oxygen uptake (VO_2) was measured using the Douglas bag method; gas fractions were analyzed by mass spectrometer (Marquette MGA 1100), and ventilatory volume was measured with either a Tissot spirometer or dry-gas meter (Collins). VO_{2max} was defined as the highest VO_2 measured from at least a 40-s Douglas bag collection. However, to verify that VO_{2max} was achieved, a supramaximal treadmill run was performed on a separate day, with the measurement of

VO_{2max} and anaerobic capacity as described below. In addition, heart rate was monitored continuously (Polar CIC, Port Washington, NY).

Supramaximal test.

On the next day following the incremental VO_{2max} test each subject performed a test to confirm the VO_{2max} value obtained in the incremental test and to estimate anaerobic capacity from the accumulated oxygen deficit, according to the method of Medbo et al. (11). Briefly, subjects ran at 8% grade with the speed chosen individually to exhaust the subject between 2 and 4 min (generally 9-10 mph for women, 11-12 mph for men). This work rate required a VO_2 that was at least 30% greater than that achieved on the incremental test. VO_{2max} was determined using the Douglas bag method in which bags were collected continuously at 45-s intervals from the start of the test. Tests were included in the analysis only if there were a minimum of three complete bags collected. Immediately after the supramaximal run, fingertip capillary blood samples were collected every 2 min for 10 min during recovery to identify the peak lactate concentration (Yellow Springs Instruments 23L, Yellow Springs, OH).

Statistics

Numerical data are presented as means $\pm$ SD. Comparisons of incremental VO_{2max} to supramaximal VO_{2max} were determined using paired t-tests. All analyses were performed with a personal computer-based analysis system (SigmaStat 2.03).

RESULTS

During the incremental test, VO_2 increased progressively with increasing work rate until the final bag, when the increment was less than half that which would be predicted from treadmill speed and grade (fig 1, solid symbols/lines). During the supramaximal test (fig 1, bars) these athletes were able to sustain this work intensity for over 3 min. Oxygen uptake increased and then plateaued, or even decreased slightly despite an aerobic work requirement at least 30% greater than VO_{2max} (solid straight line). Despite these very high and intense work rates typical of middle distance runners, no subject showed any sign or symptoms of myocardial ischemia or circulatory collapse as would be predicted if a "central governor" was acting teleologically to prevent dangerous levels of work or cardiorespiratory effort beyond that observed in the incremental test.

There was no significant difference between the VO_{2max} obtained during the incremental test (63.3 $\pm$ 6.3 ml/kg/min; mean $\pm$ SD) and that obtained during the supramaximal test (62.9 $\pm$ 6.2 ml/kg/min, N=156, p = 0.77) (fig 2). The individual data for each test (fig 2, line graphs) indicated that most individuals had almost identical VO_{2max} values for both tests, and some individuals had a supramaximal VO_{2max} value that was less then that obtained during the incremental VO_{2max} test. The peak lactate concentration measured following the supramaximal test was 12.5 $\pm$ 2.9 mmol/l; mean $\pm$ SD) confirming high rates of glycolysis to support these supramaximal work rates.

DISCUSSION

The major finding of this study was that in a large number of highly competitive middle distance runners with sufficient motivation and anaerobic capacity to stay on a treadmill at supramaximal work rates long enough to achieve a stable oxygen uptake, that this value rarely exceeded the VO_2 achieved on an incremental treadmill test, and never by a substantial amount. These data confirm convincingly the concept that there is a level of oxygen uptake in humans beyond which increases in work rate are not capable of eliciting additional increments in VO_2; this is the maximal oxygen uptake.

The classic study of Taylor, Buskirk and Henschel (19) reported in 1955 provided substantial evidence in support of VO_{2max} being a parametric measure of the cardiorespiratory system's capacity to deliver oxygen. In this investigation 115 subjects were required to perform maximal exercise tests on a motor driven treadmill in order to determine their maximal cardiorespiratory capacity to deliver oxygen. Subjects visited the laboratory on three to five separate days, at the first of which a Harvard Fitness Test (19) was given to determine the approximate maximal workload; the second consisted of a warm-up and three minutes of exercise at 7mph on a grade (determined during the prior visit) with Douglas bag gas collections for 1 minute during exercise. Each subsequent visit on successive days consisted of the grade being increased 2.5% until VO_2 differed by less then 2.1 $ml \cdot kg^{-1} \cdot min^{-1}$ which is one half to one third that expected from this increase in grade (19). The major finding of this investigation was that VO_2 increases linearly with increases in exercise workload, until a workload is reached at which the VO_2 no longer increases and plateaus with further increases in work intensity (19).

The link between the achievement of VO_{2max} and the cardiorespiratory system's ability to deliver oxygen requires that both maximal cardiac output and a maximal extraction of oxygen relative to the circulatory transit time be achieved, thereby, fulfilling the quantifiable requirement of the Fick equation (5). Subsequently, Mitchell, Sproule and Chapman (12) conducted a series of experiments requiring subjects to perform VO_{2max} tests with a protocol based upon that used by Taylor et al. (19) in order to determine the hemodynamic effects of exercise at VO_{2max}. Cardiac output (Q), systemic arterial oxygen tension (PaO_2), oxygen content (CaO_2), venous carbon dioxide content ($CvCO_2$), and hydrogen ion content (pH) were determined during maximal exercise. It was reported that at VO_{2max}, Q had increased 4.3 times and arteriovenous oxygen difference (A-V O_2 diff) had increased 2.2 times to 14.3 mL per 100 mL of blood. VO_{2max} was considered to be the value at which the measurement immediately following had a VO_2 no greater than 54 mL (the amount VO_2 rose with each increase in workload minus twice the standard deviation) (12). Subsequently, after reaching this point in the VO_{2max} exercise test, with a continued increase in work intensity Q began to decrease in all subjects while the (A-V) O_2difference continued to increase (12). These findings confirmed that VO_2 and Q had reached a maximal value, at a given workload intensity, which when the workload was increased both VO_2 and Q plateaued or decreased. Furthermore, in these subjects PaO_2 remained near resting values during the exercise, indicating that pulmonary gas exchange did not limit oxygen transport (12,13). However, it has been reported that in some athletes evidence exists that desaturation may result from a mismatch between pulmonary transit time of the blood and oxygen diffusion

capacity (4, 17, 18, 20). Therefore, in healthy non-elite endurance trained individuals the measurement of VO_2 provides a valid index of the cardiorespiratory capacity (Q_{max} x A – V O_{2diff}) to deliver oxygen.

Noakes (14,15), however, has repeatedly disputed the concept of a plateau in VO_2 being attained at maximal, and sustained at supramaximal exercise workloads. This argument is centered on the exclusion of reported supramaximal exercise results for VO_{2max}, confirming that a plateau has actually been reached, in the experiments performed by Hill and Lupton (6), Taylor et al. (19), and others who have previously studied the VO_{2max} plateau (12). A recent study (3) has demonstrated that incremental VO_{2max} is useful in exercise testing, whether or not a true plateau is reached due to a correlation between the VO_{2max} obtained during constant load exercise test and that obtained during incremental exercise. However, in the present study the data we have collected provides conclusive evidence that VO_{2max} is a quantifiable and reproducible parameter of the cardiorespiratory systems' ability to deliver oxygen to the body at a given level of physical conditioning. After analyzing the results of 156 incremental tests, we then brought each subject back to the laboratory for a supramaximal exercise test collecting a total of 156 supramaximal VO_2 tests. Each subjects' supramaximal test resulted in a VO_{2max} virtually identical to that obtained during their previous incremental maximal test, despite an aerobic work requirement that was 30% greater than that previously achieved, as well as a subsequent plateau in which the oxygen uptake did not increase above their individual VO_{2max} despite the increased work intensity. Therefore,

VO_2 reached a maximum that was not increased with supramaximal work intensity, resulting in fatigue and ultimately termination of exercise.

The purpose of this study was not to identify the mechanisms of fatigue during maximal exertion, that lead to the cessation of physical effort. However we would suggest that these data provide evidence against the concept of a central governor (16) which proposes that exercise is discontinued by the brain prior to truly maximal capacity to avoid disturbance of "homeostasis" and prevent complications of excessive effort such as myocardial ischemia. Indeed, the athletes in this study were able to perform at extremely high work rates substantially above their maximal oxygen transport capacity without untoward consequences. Such supramaximal efforts are an intrinsic part of athletic events and are performed by competitive athletes for prolonged periods of time on a daily basis.

In conclusion, VO_{2max} is an invariable parameter defining the ability of the cardiorespiratory system to transport oxygen to various tissues of the body. These data confirm and extend the previous findings of Hill and Lupton (6), Taylor et al. (19), and Mitchell et al. (12), who used VO_{2max} as an index of maximal circulatory function, despite arguments claiming it was not an accurate measurement representative of the maximal capacity of oxygen transport to the tissues. Therefore, VO_{2max} is a valid index measuring the limits of the cardiorespiratory system's ability to transport oxygen from the air to the tissues at a given level of physical conditioning and oxygen availability.

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FIGURE LEGENDS

Figure 1. Filled circles connected by lines represent Douglas bags obtained during the second minute of each 2 min stage run at a fixed speed with an increase in grade by 2% every 2 minutes. The last bag at the highest work rate was occasionally obtained earlier in the stage to accommodate subject exhaustion. Required work rate (dark abscissa) calculated directly from treadmill speed and grade. Open bars represent the last four 45 sec Douglas bags collected continuously during the supramaximal test. The light abscissa reflects the time the bags were collected during the supramaximal test. Based on running economy determined at this grade (8%), the oxygen uptake required to perform this amount of work aerobically was calculated and shown as solid line with 95% confidence limits (dashed line).

Figure 2. Comparison of mean incremental VO_{2max} (gray solid bar) and supramaximal VO_{2max} (open bar) for all 156 sets of tests. VO_{2max} data collected from each individual is represented by the line graph. Statistical analysis showed no significant difference between the incremental and supramaximal VO_{2max} results ($p = 0.77$).

Figure 1

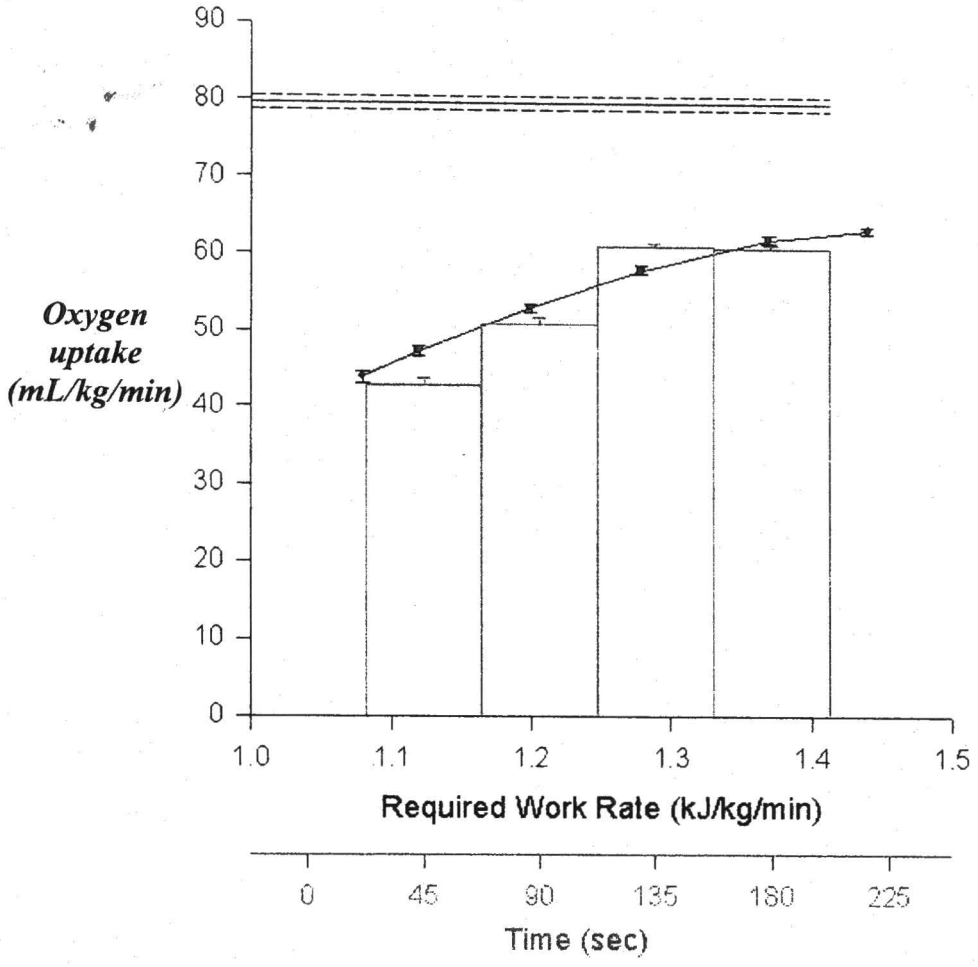
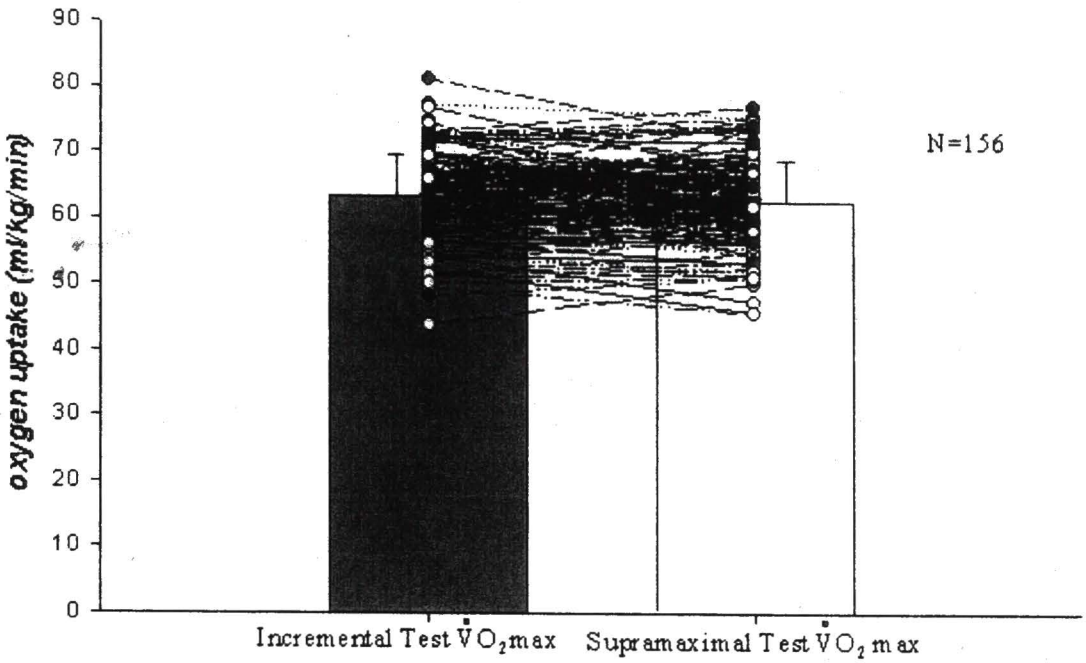

90
80
70
60
50
40
30
20
10
0
Oxygen uptake (mL/kg/min)
1.0
1.1
1.2
1.3
1.4
1.5
Required Work Rate (kJ/kg/min)
0
45
90
135
180
225
Time (sec)

Figure 2


90
80
70
60
50
40
30
20
10
0
oxygen uptake (ml/kg/min)
N=156
Incremental Test $\dot{V}O_2$max
Supramaximal Test $\dot{V}O_2$max

CHAPTER III

THE EFFECTS OF AEROBIC FITNESS AND BETA-1 ADRENERGIC RECEPTOR BLOCKADE ON CARDIAC WORK DURING DYNAMIC EXERCISE

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ABSTRACT

The purpose of this investigation was to determine whether cardiovascular adaptations characteristic of long-term endurance exercise compensate more effectively during cardioselective β_1-adrenergic receptor (βAR) blockade-induced reductions in sympathoadrenergic stimulated contractility. Endurance trained athletes (ET, n=8) and average trained (AT, n=8) subjects performed submaximal cycling exercise at moderate (45% VO_{2max}) and heavy (70% VO_{2max}) workloads with and without metoprolol. Cardiac output (Qc), heart rate (HR), and systolic blood pressure (SBP) were recorded at rest and during exercise. Cardiac work was calculated from the triple product of HR, SV, and SBP and myocardial efficiency is represented as cardiac work for a given total body oxygen consumption (VO_2). Metoprolol reduced Qc at 45% VO_{2max} ($p=0.004$) and 70% VO_{2max} ($p=0.022$) in ET subjects but did not alter Qc in the AT subjects. In ET subjects at 45% VO_{2max}, metoprolol-induced reductions in Qc were a result of decreases in HR ($P < 0.05$) and a reduced compensatory increase in stroke volume (SV, $P > 0.05$). The cardiac work and calculated cardiac efficiency were reduced with metoprolol in ET subjects at both exercise intensities and in the AT subjects during the high intensity workload ($p<0.01$). The cardiac work and the calculated cardiac efficiency were not affected by metoprolol in the AT subjects during the 45% VO_{2max} exercise trial. The βAR blockade reduced the cardiac work of the ET subjects during moderate and heavy intensity exercise but only during the heavy intensity workload in the AT subjects. We conclude that the Frank-Starling mechanism remains intact with BAR blockade in AT subjects but is impaired in ET during exercise.

Keywords: Frank-Starling mechanism, cardiac output, cardiac efficiency, acetylene rebreathe, exercise-induced cardiac hypertrophy

INTRODUCTION

Ischemic heart disease patients are routinely prescribed cardio-selective β_1-adrenergic receptor (βAR) blockers with the aim of reducing the exercise-induced increases in heart rate (HR) below the ischemic threshold for cardiac arrhythmias (35, 36). In short, by reducing the HR for a given intensity of exercise it is presumed the cardiac work required to perform the physical work would be reduced. However, it is well established that the relationship between cardiac output (Qc) and oxygen uptake (VO_2) is linear and invariant (12). Therefore, one would predict that a reduction in HR at any given work intensity would increase cardiac filling time and consequently, by reason of the Frank-Starling mechanism (11, 31), increase stroke volume (SV) and maintain cardiac work. Indeed, Joyner et al. (25) in a comparison of β-1 (Atenolol) and β-1 & β-2 (Propranolol) adrenergic receptor blockers reported similar increases in SV in endurance exercise trained (ET) and untrained (UT) men during 60% VO_{2max} treadmill exercise with βAR blockade. These results suggest that despite adaptively large cardiac preloads during exercise, ET athletes are further able to increase SV during exercise with pharmacologically-induced increases in cardiac filling time.

It has been well established, that in the ET athlete compared to UT subjects there are significant increases in eccentric cardiac hypertrophy, cardiac compliance (21, 31, 56) and circulating blood volume (7-10, 38). The observed ventricular remodeling associated with endurance exercise training (13, 15, 16, 40, 42) enables the ET athlete to continue to increase their SV during progressive increases in exercise workloads (18). Conversely, the UT subject's exercise SV plateaus at approximately 40% VO_{2max} (2, 18).

The observed plateau in SV in untrained individuals was thought to be a result of pericardial restraint (22, 51). However, the presence of a 27% increase in SV in the UT subjects performing treadmill exercise at 60% VO_{2max} with βAR blockade (25) suggests that with increased cardiac filling time the Frank-Starling mechanism remains functional and that pericardial restraint is not a factor in the initial portion of the SV plateau.

Therefore, the purpose of this study was to evaluate the effects of cardioselective βAR blockade on the ability to maintain cardiac work in average fit and high fit subjects during moderate (45% VO_{2max}) and heavy (70% VO_{2max}) intensity cycling exercise. We hypothesized that due to greater cardiac compliance, larger circulating blood volumes (2, 14, 31, 56) and the absence of a functional pericardial restraint during moderate to heavy exercise intensity (18), the ET athlete would compensate for the βAR blockade-induced reduction in myocardial contractility more effectively than average trained individuals. We tested these hypotheses by comparing the cardiac work (HR x SV x SBP) of average trained (AT) and ET subjects at 45% and 70% VO_{2max} steady state cycling exercise with and without cardio-selective β-1 adrenergic (Metoprolol) blockade.

METHODS:

Subjects: Sixteen men were recruited as volunteer subjects from the Dallas/Fort Worth metroplex area bicycle riding, triathalon, athletic and health fitness clubs. Only men were selected as subjects because women do not develop substantial increases in absolute left ventricular wall thickness in response to endurance training and have significantly smaller changes in left ventricular cavity dimensions (41). The subjects

were aged between 18 and 35 years and were free of over the counter and prescription medications. Each subject was informed of the study protocol, gave written informed consent, completed a health history questionnaire and underwent seated and standing 12-lead electrocardiography without evidence of ischemia or arrhythmia. Of the 16 volunteer subjects, 8 were endurance exercise trained (ET) competitive long distance bicyclists and runners (VO_{2max} = 62.4 $\pm$ 4.5 ml/kg/min). The other 8 subjects were involved in a consistent yet moderate aerobic fitness program and deemed average trained (AT) (VO_{2max} = 44.5 $\pm$ 4.8 ml/kg/min). Demographic and descriptive cardiac structural measures of both groups are presented in Table 1. All experimental procedures conformed to the ethical considerations as approved by the Institutional Review Board for Human Subjects of the University of North Texas Health Science Center (UNTHSC) at Fort Worth and conformed to the principles in the Declaration of Helsinki.

Experimental Protocols: Prior to performing their individual exercise stress test the subjects were examined with resting electrocardiogram (EKG) and echocardiography (Echo) in the supine position. Following their cardiac evaluation the subjects performed a graded exercise stress test on a stationary electrically braked cycle ergometer (Scifit) to volitional exhaustion in order to determine maximal oxygen uptake (VO_{2max}). The initial workload of the exercise stress test was 50 watts and each minute the workload was progressively increased dependent upon the individual subject's predicted aerobic fitness to ensure that volitional exhaustion was reached within 6-10 minutes (3). The AT subjects pedaled at 60 rpm and the ET subjects were allowed to pedal at the frequency at or above 60 rpm that they used during competition.

On a separate experimental day, at least two days after their individual maximal exercise stress test, each subject performed four steady-state 25 minute cycle exercise trials on the electronically braked cycle ergometer at the same pedal frequency that they performed their maximal exercise stress test. The first two sub-maximal exercise bouts were control trials performed at 45% VO_{2max} (moderate) and 70% VO_{2max} (heavy) exercise intensities, respectively. After reaching their required steady-state VO_2, measurements of heart rate (HR), cardiac output (Qc), and systolic (SBP) and diastolic (DBP) arterial blood pressures were obtained at five minute intervals.

After completion of the control exercise trials the subjects were allowed to recover for two hours. At the beginning of the two hour recovery period the subjects ingested 50 mg of short acting Metoprolol to enable maximal efficacy and blockade of the β-1 adrenergic receptors on the heart (55). Pharmacokinetic profiles of ingested Metoprolol identify that a peak plasma value occurs two hours after ingestion and is maintained constant for approximately another 2 to 4 hours (55).

Following the two hour recovery period a second pair of sub-maximal (45% and 70% VO_{2max}) exercise trials were performed at the individual's same pedal frequency employed during the first two trials prior to the two hour recovery period. Measurements of Qc, HR, SBP and DBP were repeated at five minute intervals. A schematic outline of the experimental protocol is presented in Figure 1.

Measurements: Supine, resting echocardiographic measurements of left ventricular mass (LVM) were obtained using Doppler ultra-sound echocardiography (Phillips HDI 5000) interfaced with a videographic recorder (Sony SVO-1410). The same

echocardiographer made recordings and measurements for each of the subjects. A 2-4 mHz probe transducer was placed between the second and fourth intercostal space lateral to the sternum. Measurements were recorded in parasternal long axis and short axis views. Left ventricular (LV) dimensions measured by M-mode echocardiography included: end-diastolic internal diameter (LVIDd), end-diastolic posterior wall thickness (PWTd), and end-diastolic interventricular septal thickness (IVSd). The LVM (g) was determined using the Penn convention (28): LVM = 1.04 x [(end diastolic diameter + PWT + IVS thickness)^3 – end diastolic diameter^3] – 13.6g.

Subjects were instrumented with a standard 3-lead EKG (Model 78342A, Hewlett Packard) for continuous monitoring of HR during cycling exercise. Oxygen uptake was continuously monitored by respiring through a mouthpiece attached to a low-resistance turbine volume transducer (Sensor Medics, VMM series) for measurement of breath volumes. Respiratory gases were continuously sampled from the mouthpiece for fractional concentrations of oxygen, carbon dioxide, and nitrogen via mass spectrometery (Perkin-Elmer MGA-1100A). Device input signals underwent analog-to-digital conversion and computer analysis (Dell Optiplex GXi) for on-line, breath-by-breath determinations. A customized software package was employed to correct for equipment delay and response times. Standardized calculations of metabolic data were corrected for ambient conditions and measurements were averaged for each workload.

In addition, Qc was measured every five minutes via acetylene rebreathe technique as described previously (53) with SV being calculated from the division of Qc by HR. Unpublished data from our laboratory verified the reproducibility of the

acetylene rebreathe technique. Ten subjects performed cycling exercise at two separate exercise intensities (40% and 60% VO_{2max}) on two separate visits to the laboratory. The order in which the exercise intensity was performed was randomized for each visit with a 2 hour break between each session to allow hemodynamic variables to return to baseline. The Qc was measured every 5 minutes at rest and during exercise. We found the technique to produce highly reproducible Qc values (r^2= 0.88) for the two visits. In addition, the average coefficient of variation for the low and high intensity workload was 7.2% and 8.4%, respectively.

Absolute SBP and DBP were recorded simultaneously with each rebreathe via an automated ambulatory arm cuff which detects the Kortokoff sounds via microphone (Suntech Tango). During treadmill exercise-ECG testing the combined mean difference between invasive and automated SBP and DBP was 4.79 $\pm$ 0.14 and 6.33 $\pm$ 0.10 mmHg, respectively (6).

The triple product of SBP, HR and SV was used as an index of cardiac work (45). Cardiac efficiency was calculated as cardiac work for a given total body VO_2.

Statistical Analysis. Demographic data is presented as mean $\pm$ standard deviation. Experimental data is presented as mean $\pm$ standard error. Paired (within groups) and unpaired (between groups) t-tests were used to determine significant differences for two variables. Analysis of variance (ANOVA) was performed when comparing multiple factors. Overall significance was further tested via post-hoc analyses using Student-Newman-Keuls test for intergroup comparison. Differences between means were

determined significant, if $p<0.05$. Analyses were conducted using SigmaStat (Jandel Scientific Software, SPSS Inc) software.

RESULTS

Evidence of endurance training in ET subjects: The subjects' demographic characteristics and LV dimensions are presented in Table 1. The values obtained for LV mass, and LV dimensions were comparable with those observed in echocardiographic studies using similar techniques on similar groups of individuals of average fitness and endurance trained competitive athletes (32, 40). The ET athletes' VO_{2max}, LV mass, LV wall thickness, and LVID were greater than AT subjects ($p<0.01$). In addition, body weights, and resting HR's of the ET subjects were less than the AT subjects ($p=0.04$, $p=0.03$, respectively).

BAR blockade induced changes in cardiovascular function during exercise: During control conditions HR, SBP, Qc, and SV increased significantly from rest to 45% VO_{2max} exercise and to 70% VO_{2max} exercise in both groups of subjects. The DBP did not change in the AT subjects during exercise or with increases in the intensity of exercise, however, the DBP of the ET subjects was reduced at both exercise workloads compared to rest and was decreased further with the increase in exercise intensity ($p<0.05$). At rest, metoprolol had no significant effect on HR in AT subjects. However, the resting HR of the ET subjects was reduced ($p<0.05$, Figure 2B). In addition, the HR's with metoprolol were reduced during exercise in both the AT and ET compared to the control (no metoprolol) exercise condition at both exercise intensities ($p<0.05$, Figure 2). The SBP

was also decreased by metoprolol in the AT and ET subjects during exercise at both workloads ($p<0.01$) but was not significantly different at rest in either group (Figure 3). Metoprolol did not change the Qc at rest or during exercise in AT ($p>0.05$, Figure 4A). However, the Qc at moderate ($p=0.004$) and heavy ($p=0.022$) intensity exercise was reduced post-βAR blockade in the ET subjects compared to control (no metoprolol) exercise (Figure 4B). In the AT subjects, SV was significantly larger after βAR blockade at rest and during both exercise workloads when compared to control (no metoprolol) conditions, $P< 0.01$ (Figure 5A). In ET subjects, metoprolol-induced reductions in Qc were a result of the decreases in HR ($P < 0.05$) and a reduced compensatory increase in SV, $P > 0.05$. However, heavy intensity exercise with metoprolol did elicit an increase in SV compared to control exercise in the ET subjects ($p=0.026$, Figure 5B). The βAR blockade did not significantly alter VO_2 or DBP at rest or during exercise conditions when compared to control.

BAR blockade effects on myocardial work at rest and during dynamic exercise: The effects of βAR blockade on cardiac work performed at rest and during moderate and intense cycling exercise workloads are summarized in Figure 6. Cardiac work was calculated from the triple product of HR, SBP, and SV. During each condition, the calculated cardiac work was greater in the ET subjects compared to the AT subjects, $p<0.05$. At rest, metoprolol did not significantly affect the cardiac work of either the AT or ET subjects. However, during moderate intensity exercise metoprolol did not attenuate cardiac work of the AT subjects when compared to control (no metoprolol) exercise at the same intensity. Conversely, metoprolol reduced the cardiac work ($p<0.01$) of the ET

subjects performing 45% VO_{2max} intensity exercise. In both groups of subjects performing heavy intensity exercise βAR blockade decreased calculated cardiac work, $p<0.01$.

The βAR blockade effects of myocardial efficiency at rest and during dynamic exercise: Cardiac efficiency is represented as cardiac work, calculated from the triple product, for a given total body VO_2. The effect of metoprolol on cardiac efficiency at rest and during exercise in the AT and ET subjects is presented in Figure 7. At rest and during the 45% VO_{2max} exercise control (no metoprolol) condition, the calculated cardiac efficiencies of the ET subjects were increased above those of the AT subjects ($p=0.05$ and $P < 0.04$, respectively). Metoprolol did not alter the calculated cardiac efficiency within either group of subjects at rest ($p>0.05$). In addition, the calculated cardiac efficiency of the AT subjects was not affected by metoprolol during the 45% VO_{2max} exercise. However, βAR blockade reduced the calculated cardiac efficiency of the ET subjects performing moderate and heavy intensity exercise, $p<0.01$.

DISCUSSION

The main finding of this investigation was an impairment of cardiac function and a reduction in the cardiac work and calculated cardiac efficiency of ET subjects performing 45% and 70% VO_{2max} exercise after cardioselective βAR blockade with metoprolol. In contrast, these same effects were not observed in the AT subjects with moderate intensity exercise. This difference appears to be related to the AT subject's ability to increase SV and maintain Qc, cardiac work and cardiac efficiency during moderate intensity exercise

despite inhibition of sympathoadrenal stimulating influences on myocardial contractility (52). However, the decreases in HR and SBP, and maintenance of DBP and VO_2 during exercise with βAR blockade in both groups of subjects confirm the findings of previous studies (1, 4, 5, 24, 25, 27, 33, 44, 47, 49, 54). The effect of βAR blockade on augmenting Qc during exercise has remained a controversial issue with some studies reporting a decrease in Qc during exercise after βAR blockade compared to control exercise (25, 27, 39), while others have reported no significant difference (4, 24, 47, 54). Many of these discrepancies are most likely a result of the pharmacological agent used to induce the cardio-selective βAR blockade, the intensity of exercise performed, the subject's VO_{2max}, and the age of the subjects.

Initially, we hypothesized that the ET subjects would be more capable than AT in maintaining cardiac work during exercise despite the βAR blockade-induced reduction in myocardial contractility. During dynamic exercise the SV of sedentary individuals plateaus at approximately 40%-45% VO_{2max} exercise (2, 18). At higher exercise intensities increases in HR and contractility become the predominate mechanisms employed to further increase Qc (43). In contrast, the adaptations elicited by endurance exercise training, such as increased myocardial compliance (31, 56), circulating blood volume (7-10, 38), and the absence of pericardial restraint (22, 51) allow the athlete to respond to exercise more effectively. Levine et al. (31) demonstrated that endurance exercise trained athletes have a greater ability to utilize the Frank-Starling law of the heart to increase Qc in the face of various ventricular volume loads at rest. Starling's law of the heart is independent of calcium-induced changes in contractility via adrenergic

stimulation of the βARs, and allows for a stretch-induced increase in force of contraction resultant of increased filling volume (19, 23). Levine et al (31) demonstrated that athletes operate on a steeper portion of the Frank-Starling curve. Therefore, for a given change in filling pressure, due to volume loading, ET athletes had a greater capacity to increase SV (31). In addition, other investigations have demonstrated athletes' enhanced ventricular filling is a result of greater use of the Frank-Starling mechanism which is more energy efficient and can further increase SV at high exercise intensities (11).

However, during βAR blockade-induced reductions in contractility, the present study has demonstrated that ET subjects were not able to maintain Qc despite increases in filling time and increased stretch of the ventricle. These results were largely due to the inability to increase SV beyond that obtained during control exercise. Conversely, Qc of the AT subjects was no different at either exercise workload with βAR blockade, largely due to significant increases in SV. This suggests the AT subject's heart is capable of increasing SV to a large enough capacity to maintain flow when filling time is increased, despite pharmacological attenuation of the effects of adrenergic stimulation. Contrary to previous investigations, (2, 18, 22, 51) these results suggest that the Frank-Starling mechanism remains intact in AT during exercise intensities above 40% VO_{2max}. Furthermore, these findings question the idea that the plateau in SV of the AT or sedentary subjects at 40%- 45% VO_{2max} is a result of pericardial restraint (22, 51). Indeed, previous data obtained from canines performing maximal treadmill exercise with and without an intact pericardium identified the presence of pericardial restraint only at near maximal exercise intensities (51). It is apparent that in ET subjects the Frank-

Starling mechanism is not as effective in increasing SV during exercise with βAR blockade, particularly at the moderate intensity workload of 45% VO_{2max}. These findings indicate that the plateau in SV of the AT subjects at 40% - 45% VO_{2max} is more likely to represent a balance between the cardiac filling time and the volume of venous return.

Metoprolol competitively inhibits neurotransmitters from binding β_1- adrenergic receptors, thereby, reducing the effects of adrenergic stimulation, such as reducing phosphorylation of proteins by protein kinase A, indirectly attenuating increases in cardiomyocyte calcium entry and a reduction in the affinity of calcium binding to the myofilaments (55). This in effect reduces HR and contractility during exercise. However, the Frank-Starling mechanism alters the force of contraction via an increase in sarcomere length. Therefore, βAR blockade-induced longer filling times would allow for greater ventricular filling and increased stretch of the contractile elements. A more forceful contraction would be re-established as a result of the increase in stretch despite the pharmacological reduction in contractility. The absence of an increase in SV in ET subjects at 45% VO_{2max} during βAR blockade indicates that the efficiency of the Frank-Starling mechanism was disrupted. Recently, it has been argued that increased stretch increases calcium sensitivity of the myofilaments, thereby, increasing the force of contraction (20, 29, 30, 50). Our results suggest βAR blockade-induced attenuation of calcium handling affects the myofilaments to a greater magnitude in ET compared to AT, specifically at moderate intensity exercise.

We also demonstrated that the cardiac work measured at rest and during control exercise of the ET subjects was greater than that of the AT subjects. This suggests that

endurance exercise training-induced increases in SV have a greater effect on cardiac work than the training induced reductions in HR. However, βAR blockade reduced the cardiac work of the ET subjects during moderate and heavy intensity exercise but only reduced the cardiac work of the AT subjects during the heavy intensity exercise trial. The reductions in cardiac work of the ET subjects were a product of significant decreases in SBP, HR, and Qc. However, in the AT the βAR blockade did not reduce the cardiac work during the 45% VO_{2max} exercise bout (figure 5). Therefore, the SV was increased sufficiently to maintain cardiac work, despite there being significant reductions in SBP and HR. These findings further support the concept that the Frank-Starling mechanism of the AT subjects remained unimpaired by βAR blockade. In addition, we calculated cardiac efficiency as the cardiac work performed (i.e. the triple product) for a given whole body oxygen uptake (37). Metoprolol, which reduces SBP (1, 4, 5, 24, 25, 27, 33, 44, 47, 49, 54) should effectively increase efficiency due to less pressure work and more volume work. However, we observed a decrease in cardiac efficiency with βAR blockade in every exercise trial compared to control, with the exception of the 45% VO_{2max} exercise of the AT subjects. This suggests that during moderate exercise intensity with βAR blockade the myocardium of the AT subjects relies less on increases in pressure and more on increases in volume to perform the same amount of work. Subsequently, the energy production necessary to perform a given amount of work was reduced and resulted in a more efficiently functioning heart (17). This would prove beneficial to patients with ischemic heart disease performing mild to moderate exercise. Metoprolol is commonly prescribed to reduce the work of the heart during adrenergic

stimulation associated with exercise without changing cardiac efficiency. However, in the present investigation, the cardiac efficiency of the ET subjects was reduced. This reduction in cardiac efficiency of the ET subjects appeared related to their inability to increase SV at the low exercise intensities.

In the present investigation a number of limitations need to be identified. A calculation of cardiac efficiency would ideally involve external cardiac work performed for a given myocardial oxygen consumption. In addition, a direct measure of myocardial oxygen consumption would have provided a more precise index of cardiac work (26). However, these measurements are highly invasive and only measure global changes in myocardial oxygen consumption without capability to isolate the LV oxygen consumption. Furthermore, the calculation of the triple product (HR x SV x SBP) has been established as a reliable index of cardiac work during changes in stroke volume r=0.926 (45). In specific situations in which the ionotropic state of the myocardium was altered the triple product calculation provided a more reliable index of cardiac work than the rate-pressure (HR x SBP) product (45). In addition, we did not challenge the efficacy of metoprolol using a selective agonist. However, according to the pharmacokinetic data of metoprolol, the peak activity of short acting metoprolol is 2-4 hours after oral administration (55). Each of our experimental exercise sessions were conducted within this time window. In addition, the reductions observed in HR, and SBP were comparable to the values reported in other studies using metoprolol and other cardioselective βAR inhibitors (1, 4, 5, 24, 25, 27, 33, 44, 47, 49, 54).

We have demonstrated that at moderate intensity exercise with metoprolol, the AT subjects were able to maintain cardiac function, cardiac work, and cardiac efficiency. The effect of workload on cardiovascular function during βAR blockade may be a result of the effectiveness of the skeletal muscle pump. Previous studies have demonstrated that the compression of the veins in skeletal muscle during contraction is more effective in increasing venous return at mild to moderate exercise workloads (34, 46, 48). The effect of the skeletal muscle pump juxtaposed with pharmacologically increased filling time, may account for the maintenance in cardiovascular function in AT with moderate intensity exercise that is dissipated at higher workloads. In contrast to our working hypothesis, βAR blockade reduced the cardiac function, cardiac work, and cardiac efficiency during moderate and heavy intensity exercise in ET athletes. These results indicate that with βAR blockade the more efficient Frank-Starling mechanism is impaired in ET athletes but remains intact and functional in AT subjects during moderate intensities of exercise.

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FIGURE LEGENDS

Figure 1. Schematic diagram of experimental protocol for control and experimental exercise trials. The Qc, SBP, and DBP were measured every 5 minutes during rest and each exercise bout. HR was monitored continuously.

Figure 2. Heart rate monitored continuously during rest, 45% VO_{2max}, and 70% VO_{2max} cycling exercise in A.) AT control condition (open circles) and post-metoprolol (closed circles) and, B.) ET control condition (open triangles) and post-metoprolol (closed triangles). HR, heart rate. * Significantly different from control in same fitness group, $P < 0.05$.

Figure 3. Systolic blood pressure measured during each rebreathe at rest, 45% VO_{2max}, and 70% VO_{2max} cycling exercise in A.) AT control condition (open circles) and post-metoprolol (closed circles) and, B.) ET control condition (open triangles) and post-metoprolol (closed triangles). SBP, systolic blood pressure. * Significantly different from control in same fitness group, $P < 0.05$.

Figure 4. Cardiac output measured every 5 minutes at rest, 45% VO_{2max}, and 70% VO_{2max} cycling exercise in A.) AT control condition (open circles) and post-metoprolol (closed circles) and, B) ET control condition (open triangles) and post-metoprolol (closed triangles). Qc, cardiac output. * Significantly different from control in same fitness group, $P < 0.05$.

Figure 5. Stroke volume calculated from Qc measured via acetylene rebreathe, and HR which was continuously monitored at rest, 45% VO_{2max}, and 70% VO_{2max} cycling exercise in A.) AT control condition (open circles) and post-metoprolol (closed circles) and, B.) ET control condition (open triangles) and post-metoprolol (closed triangles). SV, stroke volume. * Significantly different from control in same fitness group, $P< 0.05$.

Figure 6. Resting and exercise cardiac work as calculated from the triple product in AT and ET subjects pre (solid bars) and post (hatched bars) administration of metoprolol. * Significantly different from control in same fitness group, $P< 0.05$. † Significantly different from UT, $P< 0.05$.

Figure 7. Resting and exercise myocardial efficiency as calculated from cardiac work for a given total body VO_2. Data shown in AT and ET subjects pre (solid bars) and post (hatched bars) administration of metoprolol. * Significantly different from control in same fitness group, $P< 0.05$. † Significantly different from AT, $P< 0.05$.

Table 1. *Demographic variables and Echo LV measurements in ET and AT*

Variable	Average Trained	Endurance Trained	p value
Age, yr	26 ± 4.1	28 ± 4.6	0.392
Weight, kg	80.7 ± 2.9	73.8 ± 4.6	0.044
VO_{2max}, mL/kg/min	44.5 ± 4.8	62.4 ± 4.5	<0.001
LV dimensions, Parasternal Long Axis View			
IVSd, cm	0.79 ±0.1	0.94 ± 0.1	0.003
LVIDd, cm	4.9 ± 0.4	5.8 ± 0.7	0.008
PWTd, cm	0.81 ± 0.04	0.96 ± 0.1	0.004
LVM,g	131 ± 19	218 ± 42	<0.001
LVM, g/kg	1.6 ± 0.2	3.0 ± 0.5	<0.001

Values are means ± SE. VO_2, oxygen consumption; LV, left ventricle; IVSd, end-diastole intraventricular septal thickness; LVIDd, end-diastole left ventricular internal diameter; PWTd, end-diastole posterior wall thickness; LVM, left ventricle mass.

FIGURE 1

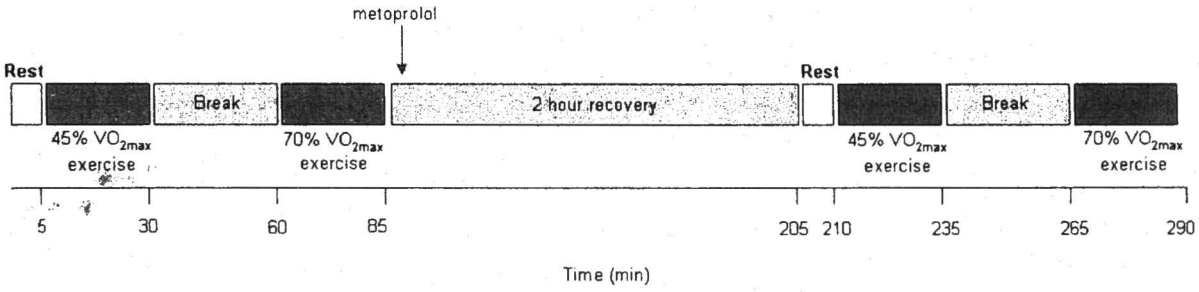

metoprolol
Rest
Break
2 hour recovery
Rest
Break
45% VO2max exercise
70% VO2max exercise
45% VO2max exercise
70% VO2max exercise
5
30
60
85
205
210
235
265
290
Time (min)

FIGURE 2

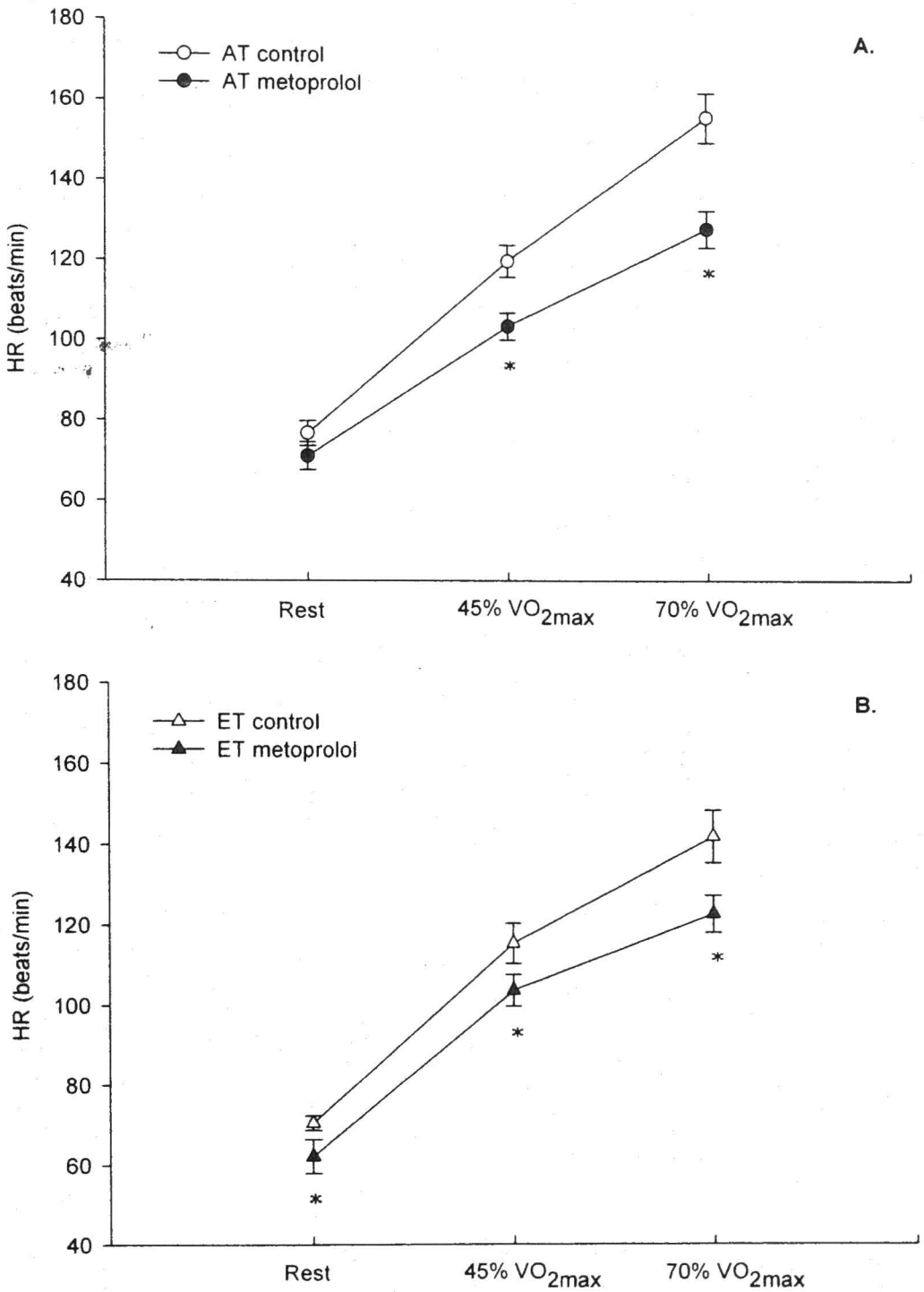

A.
AT control
AT metoprolol
HR (beats/min)
180
160
140
120
100
80
60
40
Rest
45% VO2max
70% VO2max
*
B.
ET control
ET metoprolol
HR (beats/min)
180
160
140
120
100
80
60
40
Rest
45% VO2max
70% VO2max
*

FIGURE 3

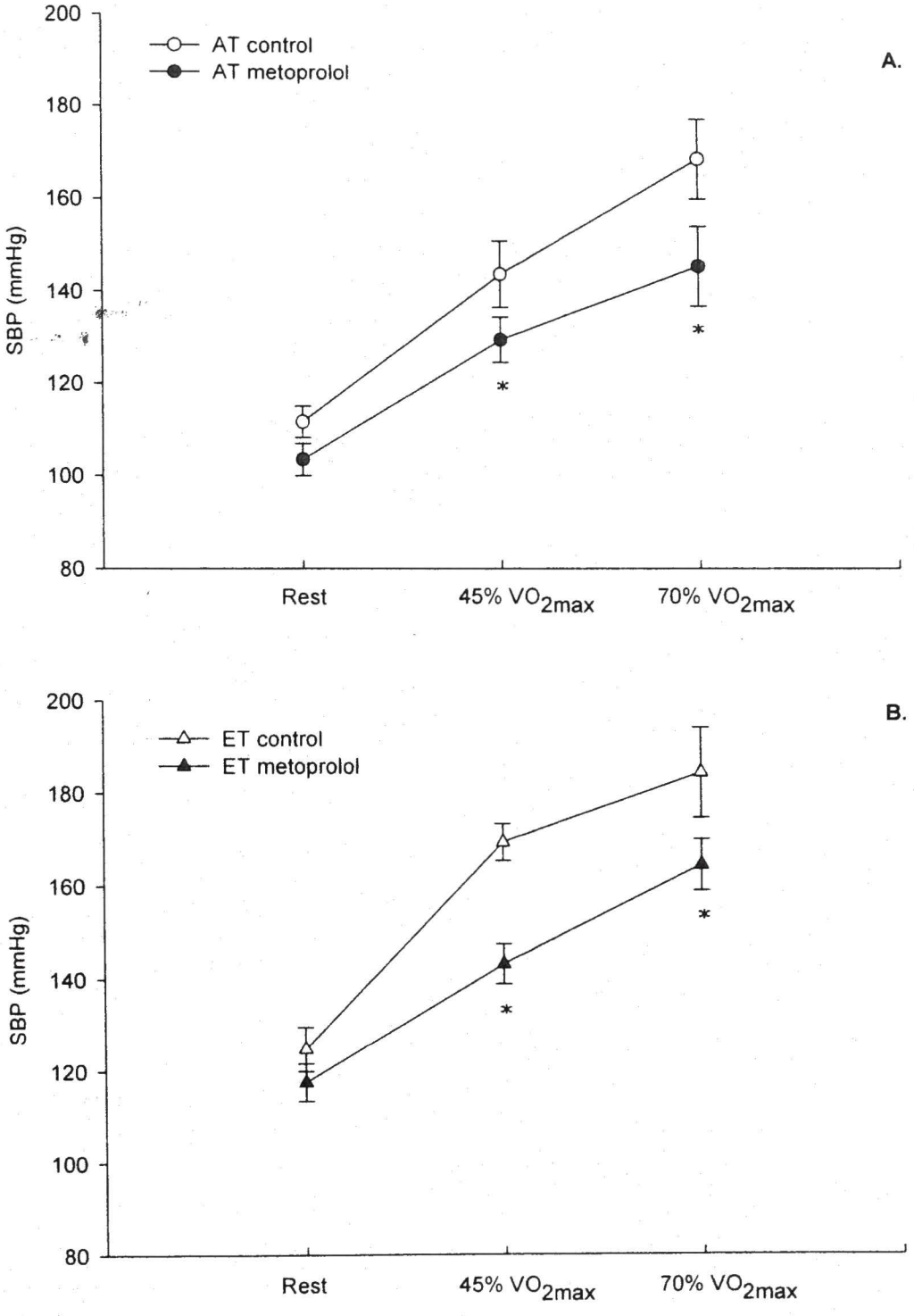
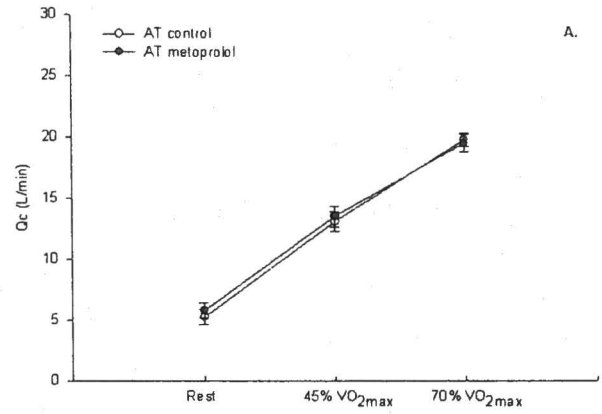

A.
AT control
AT metoprolol
SBP (mmHg)
200
180
160
140
120
100
80
Rest
45% VO2max
70% VO2max
*
*
B.
ET control
ET metoprolol
SBP (mmHg)
200
180
160
140
120
100
80
Rest
45% VO2max
70% VO2max
*
*

FIGURE 4


AT control
AT metoprolol
A.
Qc (L/min)
30
25
20
15
10
5
0
Rest
45% VO2max
70% VO2max

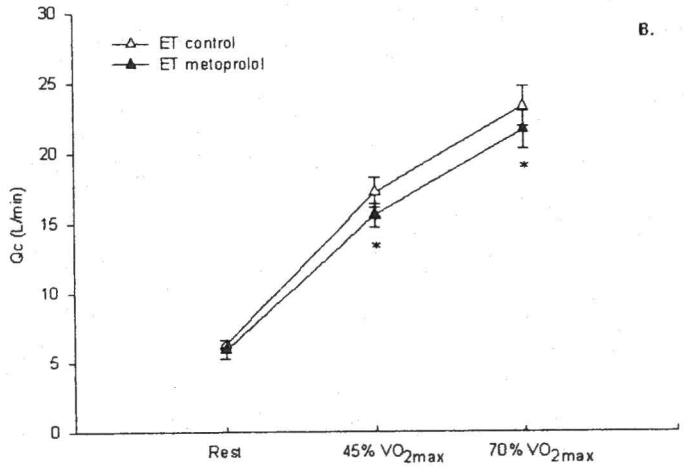

ET control
ET metoprolol
B.
Qc (L/min)
30
25
20
15
10
5
0
Rest
45% VO2max
70% VO2max
*
*

FIGURE 5

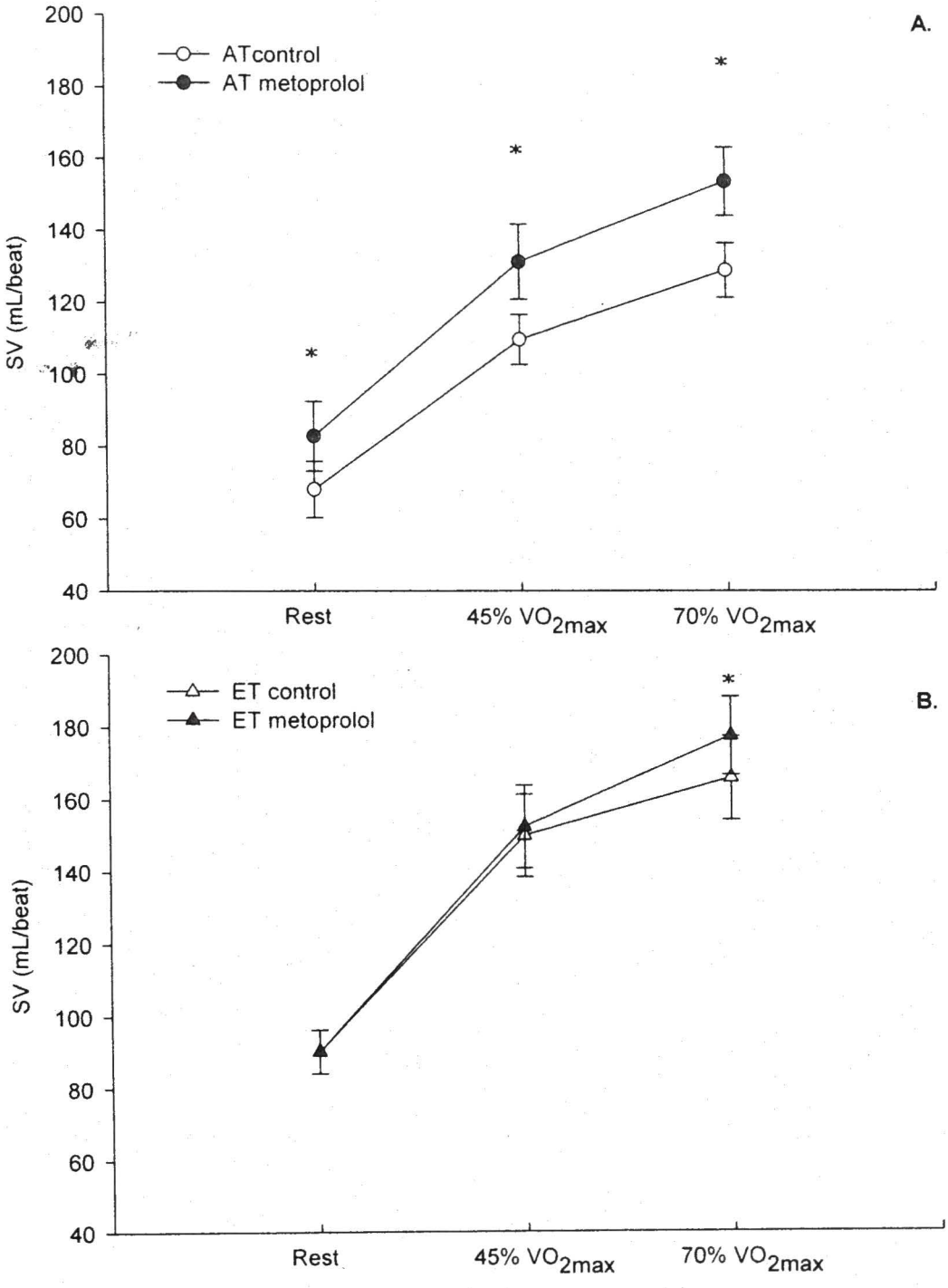

A.
ATcontrol
AT metoprolol
SV (mL/beat)
200
180
160
140
120
100
80
60
40
Rest
45% VO2max
70% VO2max
*
B.
ET control
ET metoprolol
SV (mL/beat)
200
180
160
140
120
100
80
60
40
Rest
45% VO2max
70% VO2max
*

FIGURE 6

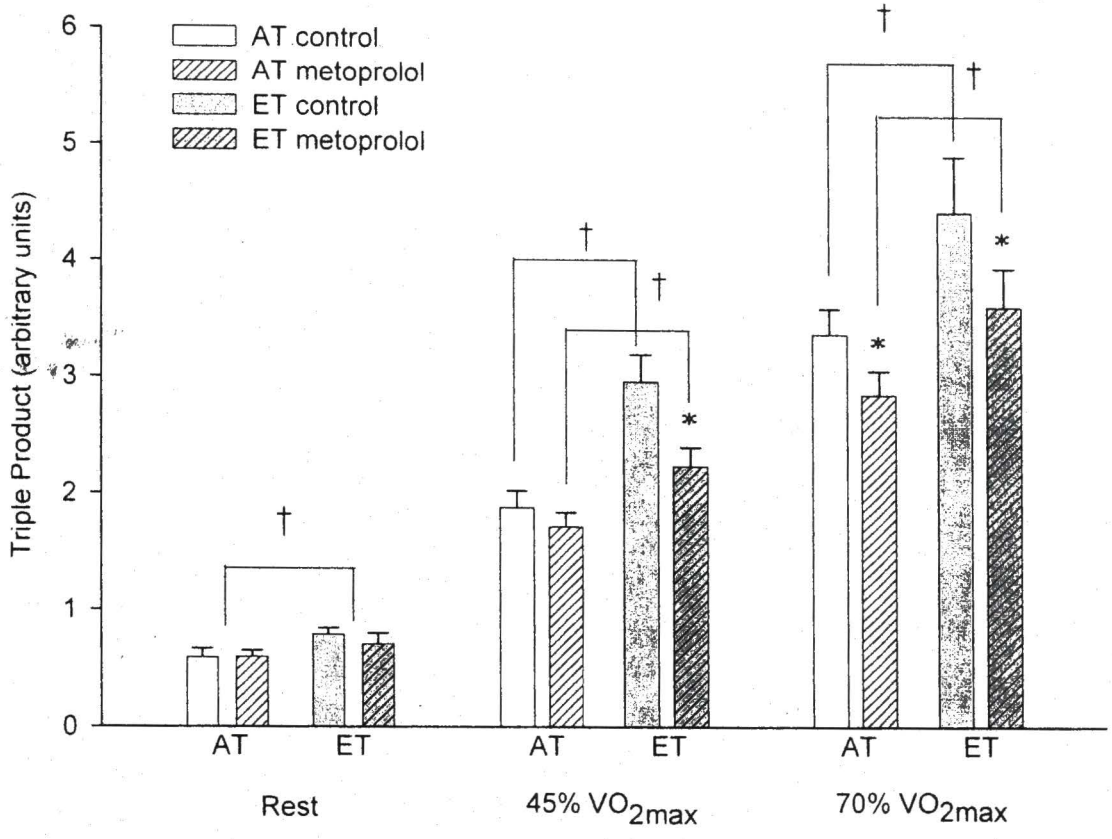
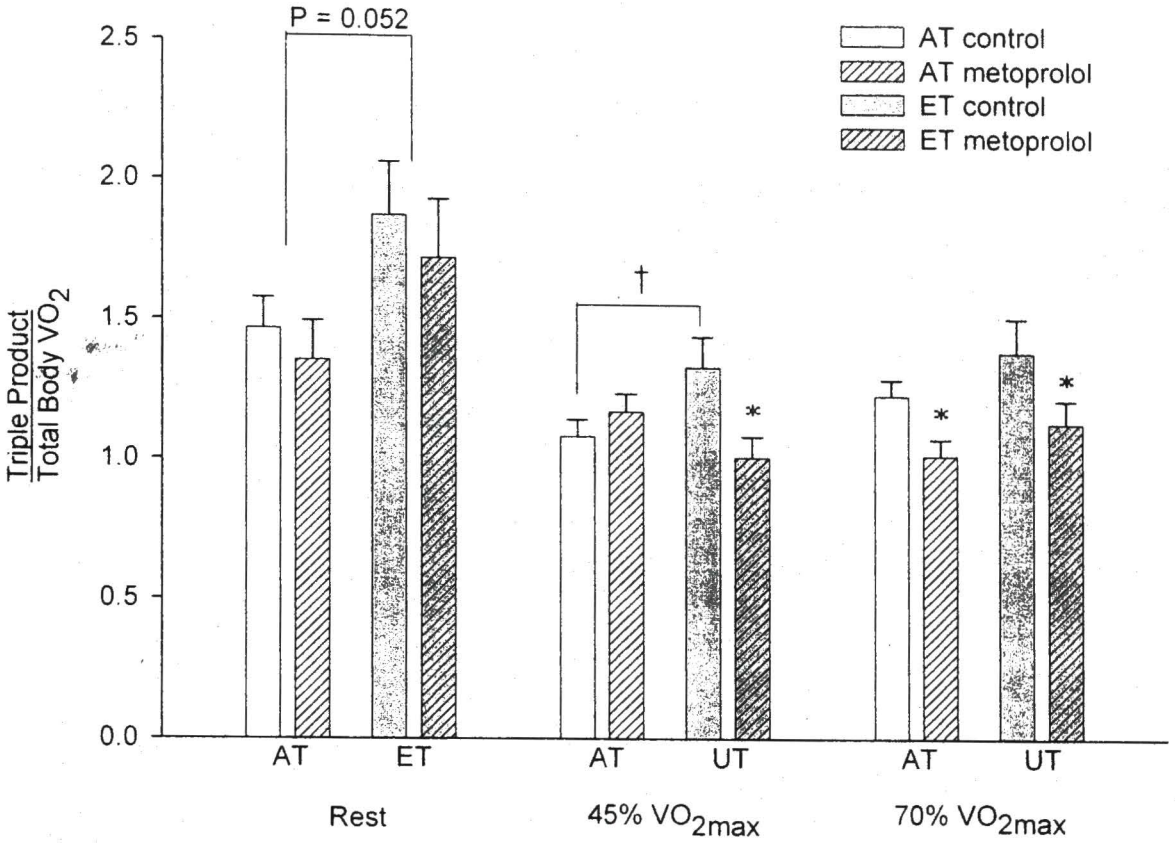

AT control
AT metoprolol
ET control
ET metoprolol
Triple Product (arbitrary units)
0
1
2
3
4
5
6
AT
ET
AT
ET
AT
ET
Rest
45% VO2max
70% VO2max
†
*

FIGURE 7


P = 0.052
AT control
AT metoprolol
ET control
ET metoprolol
Triple Product / Total Body VO2
2.5
2.0
1.5
1.0
0.5
0.0
†
*
*
*
AT
ET
AT
UT
AT
UT
Rest
45% VO2max
70% VO2max

CHAPTER IV

The effect of aerobic fitness on femoral vascular conductance and the change in central blood volume with exercise onset

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ABSTRACT

The purpose of this investigation was to determine whether endurance exercise training (ET) increased the change in central blood volume (ΔCBV) observed with exercise onset compared to average exercise trained (AT) subjects. In addition, we sought to determine if peak leg blood flow (LBF) and leg vascular conductance (LVC) were increased in ET athletes compared to AT. The ET (n=8) and AT subjects (n=8) performed a maximal exercise stress test to determine maximal oxygen uptake. The change in thoracic admittance (Δ1/TI) was measured as an index of the ΔCBV observed during the onset (10 sec) of cycling exercise. On a separate experimental day, supine LBF and LVC were measured via Doppler ultrasound technology during rest and post-ischemia induced by venous cuff occlusion and calf raising exercise. The Δ1/TI was greater in ET subjects with the onset of cycling exercise compared to AT subjects ($p < 0.05$). Resting LBF and LVC were greater in ET athletes compared to AT subjects ($p<0.01$). In addition, peak LBF's and LVC's after ischemia were larger in ET athletes compared to AT subjects ($p<0.001$). These results indicate that endurance exercise training-induced adaptations of the skeletal muscle vasculature allow for greater increases in blood flow at exercise onset, and contribute to the greater increases in central blood volume during the onset of exercise.

INTRODUCTION

Chronic endurance-exercise training results in skeletal muscle hypertrophy and angiogenesis of the skeletal muscle vasculature (13). In addition, endurance exercise training augments the conductance capabilities of the peripheral skeletal muscle vasculature (25). Venous cuff occlusion combined with heel and toe raising exercise produced a 29-fold increase in peak hyperemic calf blood flow of endurance exercise-trained subjects compared to a 19-fold rise in sedentary subjects (25). Furthermore, one-legged exercise training produced a significant increase in leg blood flow during one-legged exercise compared to pre-training (13). The increase in leg blood flow capacity was accompanied by a 20% increase in capillarization of the trained muscle vasculature (13). These adaptations ensure delivery of a sufficient amount of oxygen to a larger muscle with an increased oxygen demand during dynamic exercise.

Dynamic exercise generates skeletal muscle contractions which compress the veins thereby rapidly increasing venous outflow from the muscle and venous return to the heart and is termed "the muscle pump" (22). Moreover, the muscle pump has been proposed to enhance muscle blood flow by lowering venous pressure post-contraction and enlarging the pressure gradient across the muscles' vascular bed resulting in greater arterial inflow (9, 18). Therefore, increases in the size and blood flow capacity of the skeletal muscle vasculature may affect the function of the muscle pump by providing a pressure gradient conducive to further increases in arterial blood flow to the leg

vasculature, as well as venous flow out of the leg vasculature (14). However, the effects of chronic enduranceexercise training-induced augmentation of skeletal muscle blood flow capacity on the ability of the skeletal muscle pump to increase venous return during dynamic exercise, especially during exercise onset, has not been fully investigated.

The purpose of this investigation was to determine whether endurance exercise trained athletes (ET) exhibit a greater increase in central blood volume observed at the onset of dynamic cycling exercise compared to average trained individuals (AT). In addition, we sought to determine whether leg blood flow (LBF) and leg vascular conductance (LVC) were increased in ET athletes compared to AT subjects at rest and after an ischemic stimulus which elicits maximal vasodilatation.

METHODS:

Subjects. Sixteen men were recruited as volunteer subjects from the Dallas/Fort Worth metroplex area cycling, triathalon, athletic and health fitness clubs. These subjects were recruited for another investigation which focused on endurance-exercise training-induced physiologic hypertrophy and cardiac work (10). Only men were recruited as subjects in the previous investigation because women do not develop substantial increases in absolute left ventricular wall thickness and have significantly smaller changes in left ventricular cavity dimensions in response to endurance training (17). The subjects were aged between 18 and 35 years, and were free from over-the-counter and prescription medications. Each subject was informed of the study protocol and gave written informed

consent, completed a health history questionnaire and was screened using seated and standing 12-lead electrocardiography (EKG) without evidence of ischemia or arrhythmia. Of the 16 volunteer subjects, 8 were endurance trained (ET) competitive long distance bicyclists, runners, and triathletes (VO_{2max} = 62.4 $\pm$ 4.5 ml/kg/min). The other 8 subjects were involved in a consistent yet moderate aerobic fitness program and deemed average trained (AT, VO_{2max} = 44.5 $\pm$ 4.8 ml/kg/min). Demographic variables are presented in Table 1. All experimental procedures conformed to the ethical considerations as approved by the Institutional Review Board for Human Subjects of the University of North Texas Health Science Center (UNTHSC) at Fort Worth and conformed to the principles of the Declaration of Helsinki.

Experimental Protocol. Prior to participation in the study protocol each subject performed a graded exercise stress test on a stationary electrically braked cycle ergometer (Scifit) to volitional exhaustion in order to determine their maximal oxygen uptake (VO_{2max}). The initial power of the exercise stress test was 50 watts and each minute the power was progressively increased dependant upon the individual subject's predicted aerobic fitness to ensure that volitional exhaustion was reached within 6-10 minutes (2). The AT subjects pedaled at 60 rpm and the ET subjects were allowed to pedal at the frequency at or above 60 rpm they used during competition. These data have been reported previously (10).

On a separate experimental day, supine measurements of LBF, LVC and femoral artery diameter (FAD) were assessed with M-mode Doppler ultrasound at rest and post-ischemia induced by venous cuff occlusion above the knee. Mean arterial blood pressure

(MAP) was continuously monitored via finger photoplethysmography. Subjects were placed in a supine position with the leg raised 20-25° to facilitate venous drainage with a 26 inch occlusion cuff (Zimmer, Aspen Labs) placed directly above the knee. The FAD and femoral blood velocity (FBV) were recorded simultaneously at rest. The occlusion cuff was then inflated to a suprasystolic value of 200 mmHg. Occlusion was validated by imaging the ablation of popliteal artery blood flow post-cuff inflation. Subjects then stood and performed heel raising exercise to voluntary fatigue which took an average of 110 ± 7 seconds. The subject then returned to the supine position with leg elevated 20-25° and the cuff was released. The FAD and FBV were recorded simultaneously for 1 minute post-cuff release. This procedure was repeated three times and the velocity measurements for 10 beats during rest and the first 10 beats of the plateau phase (4 – 5 sec after cuff release) were averaged for presentation of mean rest and peak velocity data.

On another experimental day, subjects performed cycling exercise at the same pedal frequency performed during the maximal exercise stress test (insert rpm here). Thoracic impedance (TI) was measured while sitting at rest on the bike and during the first 30 seconds of cycling exercise at 50 W. Changes in thoracic admittance (Δ1/TI) were used as an index of changes in central blood volume (CBV). The Δ1/TI were calculated by comparing 1 min segments taken immediately preceding exercise and during the first 30 sec of exercise at 50W. A schematic outline of the experimental protocol is presented in Figure 1.

Measurements. Supine measurements of FAD and FBV were obtained at rest, and post-ischemia using continuous wave 2-D and M-mode Doppler ultra-sound (Phillips HDI

5000) interfaced with a videographic recorder (Sony SVO 1410). The same sonographer made recordings and measurements for each of the subjects with a linear array, duplex transducer placed 2-3 cm above the bifurcation of the common femoral artery (19). When obtaining velocity measurements a correction for the external angle of insonation was performed continuously using the guidance of the longitudinal 2D image of the femoral artery in high-pulsed frequency mode together with real-time 2D vessel imaging (19). Resting and peak LBF (LBF*rest*, LBF*peak*) was calculated from an average of 10 beats of FBV and FAD measurements at rest and 4-5 seconds after release of the cuff, respectively, using the following equation:

$$\text{LBF} = \text{FBV} * \pi * \text{radius of the femoral artery}^2.$$

Beat-to-beat arterial blood pressure was measured non-invasively by a servo-controlled finger photoplethesmogram (Finometer, Finapres Medical Systems, Amsterdam, NL) placed on the middle finger of the left hand maintained at heart level in the supine position. Using this method, changes in mean arterial pressure (MAP) were recorded at rest and post-cuff occlusion and have previously been demonstrated as being no different from direct ABP measurements at rest and during dynamic exercise (11). The leg vascular conductance at rest (LVC*rest*) and post-ischemia (LVC*peak)* was calculated using the following equation:

$$\text{LVC} = \text{femoral blood flow} / \text{mean arterial pressure},$$

with the respective rest and peak LBF measurements.

During cycling exercise subjects were instrumented with a standard 3-lead EKG (Model 78342A, Hewlett Packard) for continuous monitoring of HR. Oxygen uptake was

continuously monitored by respiring through a mouthpiece attached to a low-resistance turbine volume transducer (Sensor Medics, VMM series) for measurement of breath volumes. Respiratory gases were continuously sampled from the mouthpiece for fractional concentrations of oxygen, carbon dioxide, and nitrogen via mass spectrometery (Perkin-Elmer MGA-1100A). Standardized calculations of metabolic data were corrected for ambient conditions and measurements were averaged for each workload. Impedance cardiography (Minnesota Impedance Cardiograph, Model 304B) was used to measure thoracic electrical impedance (TI). Changes in thoracic admittance ($\Delta 1/TI$) between a high and low frequency reflect the distribution of erythrocytes in the body and can be used as an index of ΔCBV as previously described (5). All signals were interfaced with a personal computer equipped with customized data acquisition software for the beat-to-beat recording of physiological variables. The EKG signal, the arterial pressure waveforms and TI were sampled at 1 kHz and real-time beat-to-beat values of HR, MAP and TI were stored for offline analysis.

Statistical Analysis. Mean differences between groups were determined by one-way Analysis of Variance and significance was set at the 0.05 level of confidence. In order to compare experimental groups during multiple conditions a two-way analysis of variance was performed. Overall significance was further tested via post-hoc analyses using Student-Newman-Keuls test for intergroup comparison of the means. Differences between means were determined significant if $p < 0.05$. Analyses were conducted using SigmaStat (Jandel Scientific Software, SPSS Inc) software.

RESULTS

Demographic characteristics of the subjects are presented in Table 1. The AF and HF groups were significantly different in VO_{2max} and body weight.

Change in Central Blood Volume at Exercise Onset: The ΔCBV was measured at rest and at the onset of 50 W cycling exercise in AT and ET subjects. The ΔCBV from rest to exercise was significantly greater in ET subjects than that of AT subjects ($p<0.05$, Figure 3).

Femoral Blood Flow and Femoral Vascular Conductance

The MAP, LBF, and LVC measured at rest and post-venous cuff occlusion with exercise are presented in Table 2. Due to an inability to maintain an acceptable image of the femoral artery the data of two AT subjects and one ET subject were not used. Resting measurements of LBF, and LVC measured were significantly different between AT and ET subjects 51.8 ± 1.1 cm^3/sec and 84.6 ± 10.6 cm^3/sec ($p = 0.015$); and 0.662 ± 0.058 cm^3/sec/mmHg and 0.963 ± 0.107 cm^3/sec/mmHg ($p< 0.01$), respectively (Figure 4A, 4B). Resting MAP was not significantly different between the AT (78.7 ± 3.7 mmHg) and ET (82.9 ± 2.3 mmHg) subjects. The time of heel raising exercise performed during venous cuff occlusion was no different between groups and averaged approximately 2 minutes. Peak LBF and LVC were higher compared to rest in ET subjects ($p<0.001$) (Fig 4A, 4B). In AT subjects, LBF*peak* was significantly higher compared to rest. The LVC*peak* in AT subjects (1.047 ± 0.083 cm^3/sec/mmHg) was less that that of the ET subjects (1.515 ± 0.117 cm^3/sec/mmHg, $p < 0.001$). In addition, LBF*peak* was greater in ET subjects (141.1 ± 11.7 cm^3/sec, $p < 0.001$) than AT subjects (86.1 ± 3.2 cm^3/sec).

The change in LVC from rest to peak was greater in ET compared to AT subjects, $p = 0.07$. The MAP post-ischemia was increased in AT compared to their respective resting measurements ($p = 0.012$), however MAP was not significantly different in the ET after venous cuff occlusion. Furthermore, MAP post-ischemia was no different between the AT and ET subjects (92.4 ± 2.7 mmHg and 88.1 ± 4.2 mmHg, respectively.

DISCUSSION

The aim of this investigation was to test the hypothesis that skeletal muscle vascular adaptations induced by chronic endurance exercise training augments leg vascular conductance (LVC) and the increase in central blood volume (CBV) observed with the onset of exercise. The findings from this investigation demonstrate: i) that the ΔCBV observed with the onset of exercise is significantly larger in ET compared to AT subjects; and ii) that LBF and LVC measured directly from the femoral artery were significantly greater in ET subjects compared to AT individuals at rest and post-ischemia.

The onset of exercise elicits a rapid increase in CBV in AT and ET individuals (19, 22). Metabolic mechanisms have been attributed to the sustenance of muscle blood flow throughout a dynamic exercise session, however, the mechanism responsible for increasing blood flow immediately with exercise onset remains controversial (4, 7, 21-23, 26, 27). The augmentation of skeletal muscle perfusion during dynamic exercise, particularly the onset of exercise, has been heavily debated centering around two major mechanisms, the skeletal muscle pump and metabolic vasodilation. The muscle pump is defined as the mechanical activity of muscle contraction and relaxation affecting the

arteriovenous pressure gradient, thereby imparting energy on blood and increasing both venous return and arterial inflow to the active muscle's vascular bed (26). It has been proposed that dynamic exercise, particularly at low and moderate intensities (6), is more likely to rely on muscle pump function rather than static muscle contraction due to the recruitment of a greater number of skeletal muscle fibers (14). In addition, the rate of muscle contraction with dynamic exercise alters the effectiveness of muscle pump function (6, 16). Similar to other investigations (19, 22) we have demonstrated that CBV is increased rapidly upon onset of exercise (Figure 2). The increase in thoracic admittance (Δ1/TI), an index of the ΔCBV (5), from rest to dynamic cycling exercise at a low workload is significantly greater in ET subjects compared to AT subjects (Figure 3). These data indicate that the change in venous return upon the onset of exercise is greater in ET subjects compared to the AT. Furthermore, the greater Δ1/TI of the ET subjects is evident within the first seconds, or muscle contractions of cycling exercise (Figure 2), and is, therefore, likely due to enhanced activation of the skeletal muscle pump.

Radegran and Saltin (19) demonstrated that skeletal muscle blood velocity, measured at the femoral artery, was significantly increased with the first relaxation of passive and voluntary exercise. They further concluded that the increase in blood flow during the first few seconds of exercise was likely due to mechanical factors (ie. muscle pump function) (19). In addition to our data, previous investigations have also demonstrated that peripheral vascular blood flow measurements were greater in trained compared to sedentary subjects at rest and during various vasodilatory challenges (13, 20, 25). Furthermore, MAP was not significantly different between resting and peak FBF

measurements. These data suggested that the increased blood flow in response to a greater oxygen demand of the tissues at the start of dynamic exercise was related to structural adaptations of the skeletal muscle vasculature. An increase in capillarization of the skeletal muscle tissue would decrease resistance and allow a greater resting flow to the tissues to without altering pressure. Indeed, Klausen et al. (13) demonstrated that one-legged endurance exercise training increased capillarization of the trained skeletal muscle. Furthermore, total peripheral resistance and leg vascular resistance were reduced during submaximal and maximal dynamic one-legged exercise (13). In an ET athlete with a larger skeletal muscle vascular bed and resting LVC, the onset of cycling exercise would result in a reciprocal increase in venous return. In addition, chronic endurance exercise training results in a larger circulating blood volume (8). A greater volume available to re-fill the venous system during relaxation of the working skeletal muscle would consequently increase intramuscular pressure even further. Compression of the veins upon the next muscle contraction would result in a larger pressure gradient and further increasing the arterial inflow with the next contraction. Furthermore, it has been demonstrated that endurance exercise training elicits morphologic changes in muscle fiber type increasing the amount of type I and type IIA red, oxidative muscle fibers (1, 3, 12, 24). In addition, recruitment of red, oxidative muscle fibers have been demonstrated to elicit further increases in blood flow and conductance during rhythmic contractions than recruitment of white, glycolytic muscle fibers (15). Type I and Type IIA muscle fibers characteristically have more mitochondria, and a greater oxygen demand. This suggests that the type of muscle fiber recruited during dynamic exercise may increase the

oxygen demand of the tissue and metabolically enhance the response of the vasculature by increasing muscle blood flow.

In summary, the present data demonstrated that the increase in CBV observed during the onset of exercise was significantly greater in endurance exercise trained athletes compared to average trained individuals. In addition, resting and peak LVC was larger in ET subjects than AT subjects. These data indicate that endurance exercise training induced adaptations of the skeletal muscle vasculature to allow for a greater conductance of blood flow through the vasculature at exercise onset.

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FIGURE LEGENDS

Figure 1. Schematic diagram of experimental protocol for Visits 1 and 2. During visit 1 leg blood flow (LBF) measurements were made via Doppler Ultrasound technology. During visit 2 thoracic impedance was measured via impedance cardiography at rest on a cycle ergometer, and during 1 minute of cycling exercise at 50 W. Thoracic admittance (1/TI) was used as an index of change in central blood volume (ΔCBV)measurements during cycling exercise. These data were collected during the previously reported investigation using the same subjects (10)

Figure 2. Sample of a 10 sec thoracic impedance (TI) recording measured during rest and with the onset of cycling exercise at 50W. The top tracing is an individual average trained (AT) subject. The bottom tracing is an individual endurance exercise trained (ET) subject.

Figure 3. Changes in thoracic admittance (Δ1/TI) in the progression from rest to the first 10 seconds of cycling exercise at 50 watts in average trained (AT) and endurance exercise trained (ET) subjects. Thoracic admittance and is used as an index of central blood volume. Δ1/TI, change in thoracic admittance. * Significantly different from average trained subjects, $p<0.05$.

Figure 4. Leg blood flow (LBF) and leg vascular conductance (LVC) measured at rest and after ischemia induced by venous cuff occlusion in conjunction with calf raising exercise. A.) rest (black bars) and peak (gray bars) LBF in AT and ET subjects, B) rest (black bars) and peak (gray bars) LVC in AT and ET subjects. The change in LVC from rest to peak was greater in ET compared to AT, $p = 0.07$. *Significantly different from rest, $p<0.05$. ** $p<0.001$. † Significantly different from AT subjects, $p<0.05$.

Table 1. *Demographic variables in endurance trained and average fit subjects*

Variable	Average Trained	Endurance Trained	p value
Age, yr	26 ± 4	29 ± 4	0.153
Weight, kg	81 ± 3	72 ± 5	0.037
VO_{2max}, mL/kg/min	44.5 ± 4.8	61.8 ± 4.5	<0.001

Values are ± SE. VO_{2max}, maximal oxygen uptake.

Table 2. *Hemodynamic response to venous cuff occlusion and heel raising exercise in endurance trained and average trained subjects*

Variable	Average Trained	Endurance Trained	p value
MAP*rest*, mmHg	79 ± 9	83 ± 6	0.388
MAP*peak*, mmHg	92 ± 7	88 ± 11	0.375
FBF*rest*, cm/sec	52 ± 3	85 ± 28	0.015
FBF*peak*, cm/sec	86 ± 8	141 ± 31	<0.001
FVC*rest*, cm^3/sec/mmHg	0.67 ± 0.11	1.06 ± 0.31	0.006
FVC*peak*, cm^3/sec/mmHg	0.92 ± 0.05	1.60 ± 0.27	<0.001

Values are ± SE. MAP, mean arterial pressure; LBF, leg blood flow; LVC, leg vascular conductance.

Figure 1

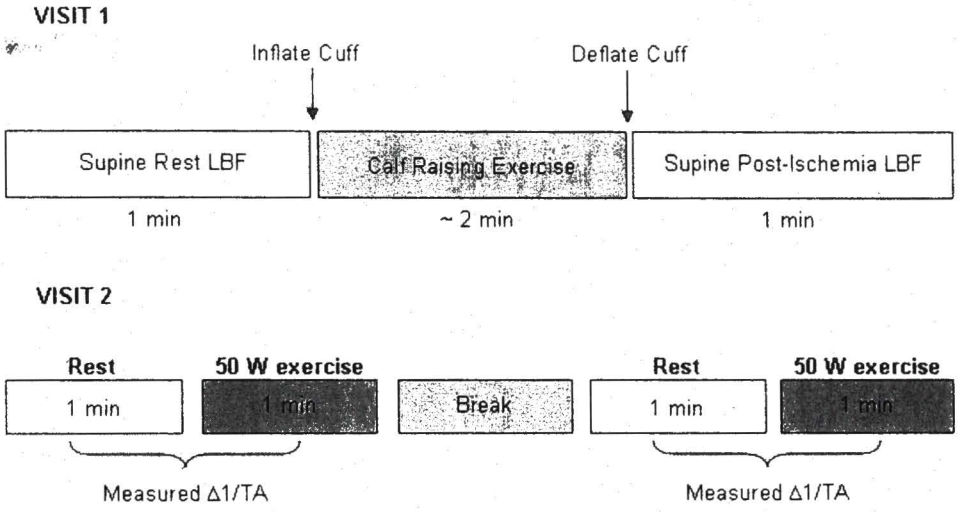

VISIT 1
Inflate Cuff
Deflate Cuff
Supine Rest LBF
Calf Raising Exercise
Supine Post-Ischemia LBF
1 min
~ 2 min
1 min
VISIT 2
Rest
50 W exercise
1 min
1 min
Break
Rest
50 W exercise
1 min
1 min
Measured Δ1/TA
Measured Δ1/TA

Figure 2

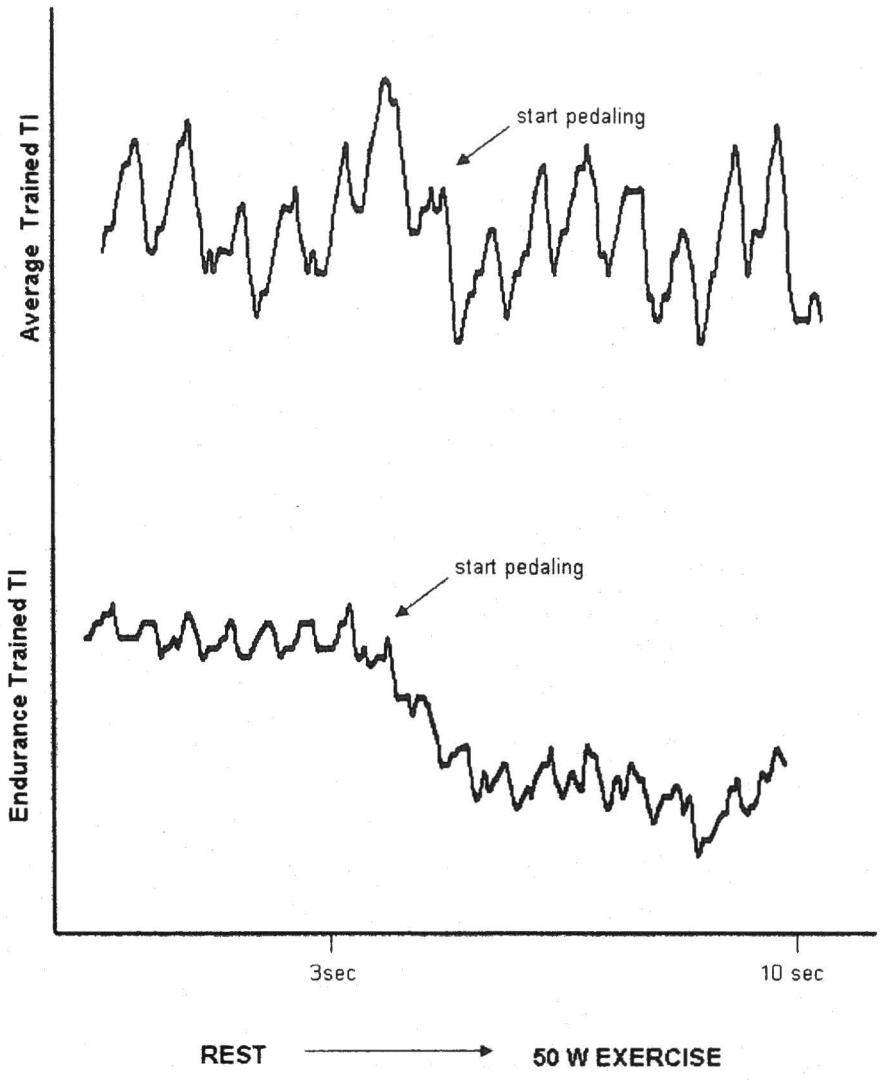

Average Trained TI
start pedaling
Endurance Trained TI
start pedaling
3sec
10 sec
REST
50 W EXERCISE

Figure 3

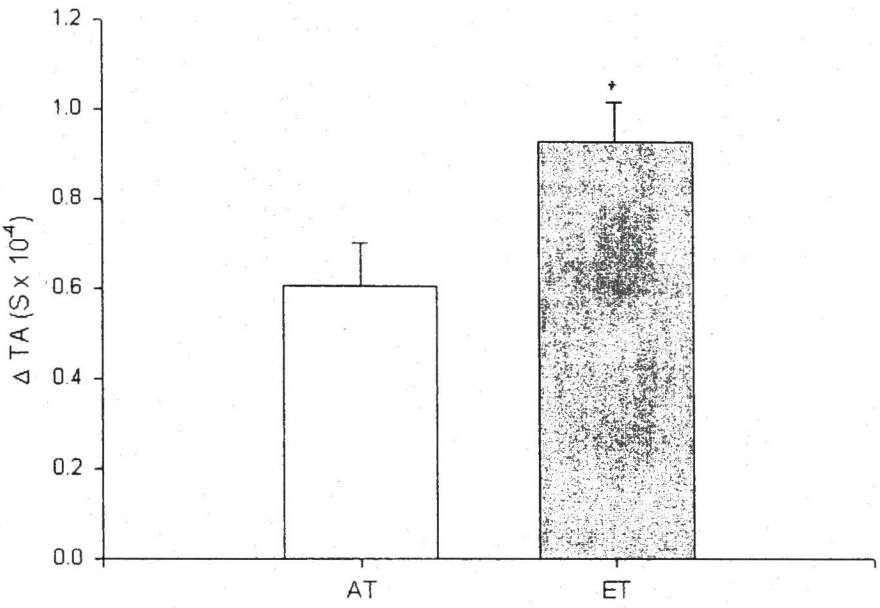
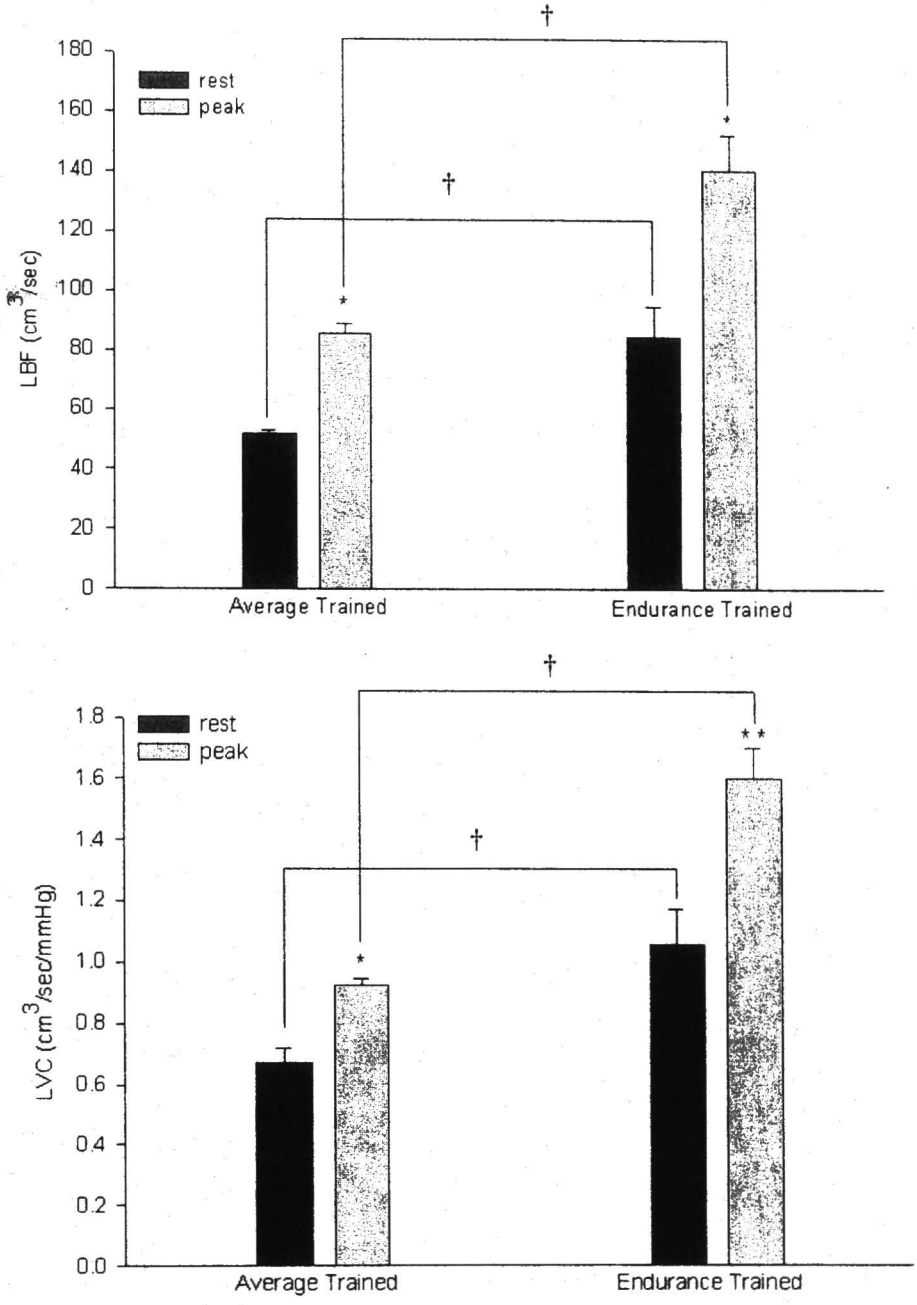


Figure 4.


†
†
rest
peak
LBF (cm 3/sec)
180
160
140
120
100
80
60
40
20
0
*
*
Average Trained
Endurance Trained
†
†
rest
peak
LVC (cm 3/sec/mmHg)
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
*
**
Average Trained
Endurance Trained

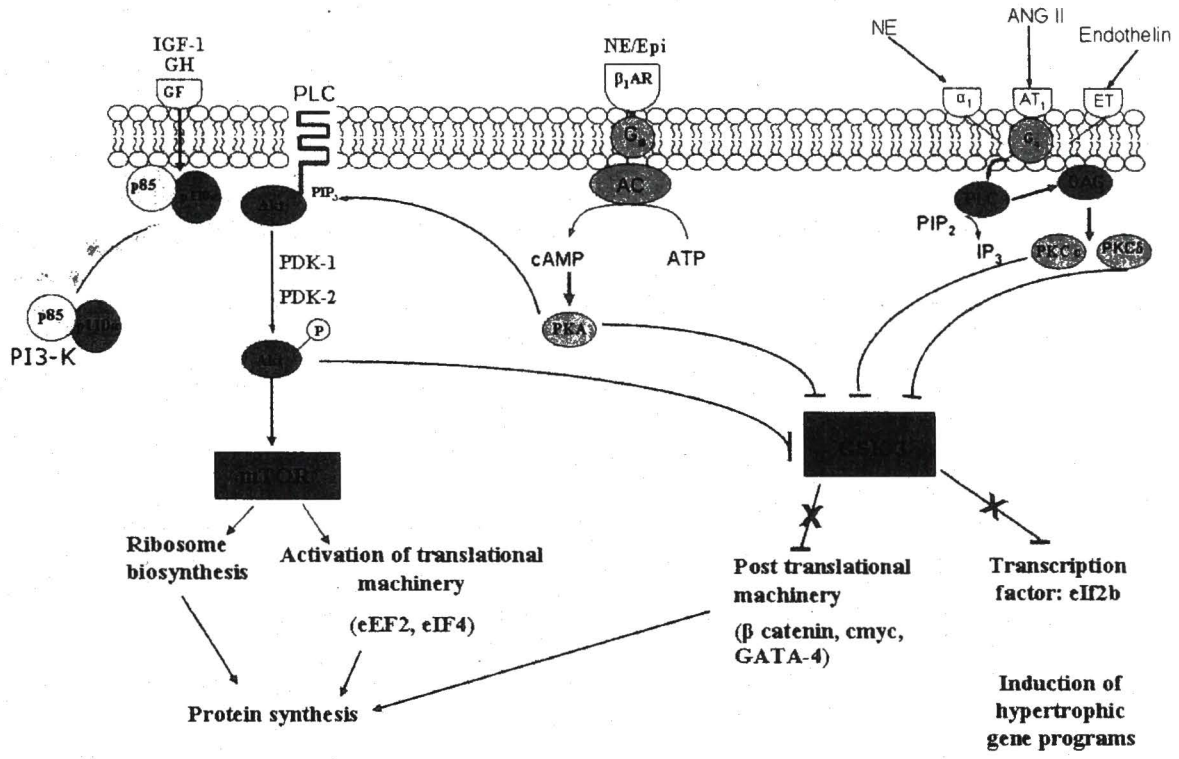
CHAPTER VI

SUGGESTED FUTURE RESEARCH

Many questions remain unanswered regarding long-term treatment with cardioselective βAR blockade and the investigations presented in this dissertation have raised additional questions as to the acute effects of βAR blockade on endurance trained (ET) and average trained (AT) individuals. Listed below are suggestions for future research intended to address specific questions that would expand the work of this dissertation, as well as the knowledge pertaining to the effect of cardioselective sympathetic inhibition on endurance training-induced cardiovascular adaptations and cardiac function.

I. To expand our understanding of the effects of long-term βAR blockade on cardiac adaptations to a rehabilitative exercise training program, it would be valuable to develop an experimental model that incorporates longitudinal measurements of left ventricle dimensions, cardiac function and exercise capacity in subjects involved in an exercise training program in conjunction with βAR blockade therapy. Various animal studies have identified the sympathetic nervous system as a common link between exercise training and physiologic cardiac hypertrophy. Repeated stimulation of βAR, characteristic of a chronic exercise routine has been directly implicated in the activation of signaling pathways that elicit favorable hypertrophic gene programs in rats; however,

this role has yet to be investigated in humans. Figure 1 provides a diagram of the proposed molecular signaling pathways and their possible involvement in the development of physiologic cardiac hypertrophy.



Physiologic Cardiac Hypertrophy

Endurance exercise training takes approximately 3 months to develop to a significant degree and the eccentric hypertrophy evolves significantly within 6 months. Therefore, in order to discern the effects of the βAR signaling pathway a longitudinal study of exercise training in which participating subjects take a cardioselective βAR blocker prior to every exercise session would be expected to elucidate the effects of this receptor pathway on the development of physiologic cardiac hypertrophy.

II. To further examine the role of myocardial oxygen consumption and myocardial efficiency in endurance trained compared to average fit subjects. We were limited in our investigations as to how mVO_2 could be measured. However, it would be interesting to measure mVO_2 via PET scanning technology and a tracer that readily diffuses into the myocardial tissue. In addition, left ventricle mass measurements at rest made by MRI technology would add to the accuracy of the investigation. Cardiac efficiency could then be calculated directly from measurements of external work and mVO_2 for a given cardiac mass, and compared in sedentary, average fit, and endurance trained subjects to determine the effects of physiological cardiac hypertrophy on cardiac efficiency.

III. To further assess the possible role of an increased skeletal muscle pump function in endurance exercise trained compared to average fit subjects. We have demonstrated that exercise trained subjects have a greater increase in central blood volume upon exercise onset. However, we cannot say for certain whether this is related to an increase in skeletal muscle pump function. An experimental design to test this hypothesis could include femoral artery blood velocity measurements during cycling exercise, or one-legged kicking exercise, in addition to measures of thoracic impedance. Invasive measurements of central venous pressure and or stroke volume via thermodilution would add further support to the increase in stroke volume resultant of blood expulsion from the exercising muscle.

APPENDIX

Acetylene Rebreathe Technique for Determining Cardiac Output

The acetylene rebreathe technique is a noninvasive respiratory technique which employs the use of an inert soluble gas, acetylene (C_2H_2), to indirectly measure pulmonary capillary blood flow. The calculation of cardiac output (Qc) via an indirect Fick method is based upon the rate of alveolar-capillary transfer of a soluble gas being proportional to pulmonary capillary blood flow which is equal to Qc in healthy humans. When C_2H_2 is inhaled its partial pressure in the blood of the pulmonary capillary bed is proportional to the partial pressure in the alveoli (3). The change in the amount of gas in the lungs is derived from the measured changes in alveolar volume and gas concentration at the end of the rebreathe. It is assumed that the gas concentration at end-expiration is equal to the concentration to which the pulmonary blood is exposed (3). Helium, an inert insoluble gas is also added to the system to provide a reference point for changes in lung volume and alveolar dead space. Once these measurements have been corrected for, the initial alveolar C_2H_2 concentration can be estimated after a single breath of gas mixture containing the two gases (1). During a rebreathe procedure the helium concentration plateaus after the third expiration indicating a sufficient mixing of the inhaled gas, including C_2H_2, within the dead space and residual volume of the lung. However, the slope of C_2H_2 is downward due to continuous uptake of

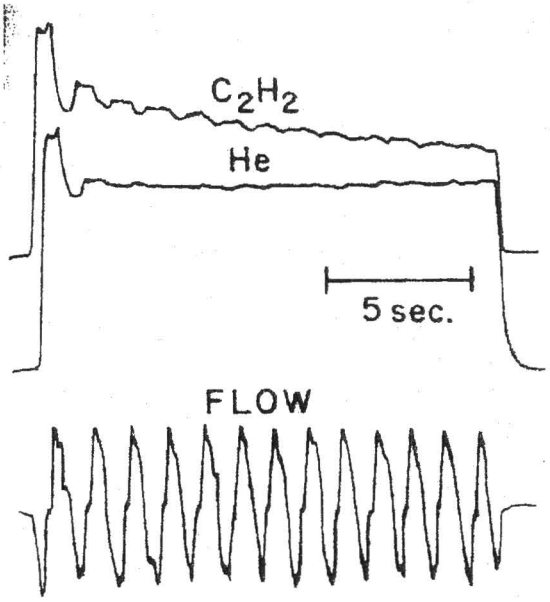
acetylene by the pulmonary capillary blood moving through the lung. This blood flow through the lung is the Qc. The computer program samples the end-respiratory gas concentration during each breath and the slope of the change in C_2H_2 concentration over time is calculated. The following calculation is used to derive the Qc (3).

$$Qc = \frac{\{(Vs/int) \times [760 /(B-470]\}}{0.700} \quad x \ -m$$

Where Vs is the system volume, int is related to the volume of the rebreathing system and tissue volume and –m is the linear regression analysis and determination of the slope of the concentration ratio of C_2H_2 over time (3). The acetylene rebreathe technique has been demonstrated to be highly correlated with direct Fick measurements of cardiac output via indicator dilution techniques at rest and during exercise (2, 3)

In our custom made rebreathe system the subjects inspire a breath from a tank containing a gas mixture of 0.5% acetylene, 9% helium, 36% oxygen, and 54.5% nitrogen. The subjects then re-breathe the gas mixture into a 5L bag for 4-6 breaths. A mass spectrometer (Perkin-Elmer MGA-1100A) continuously monitors the progressive decrease in the concentration of acetylene in the bag and a customized computer program (HEX-3) calculated the rate of disappearance. The computer program was programmed with the diffusion rate and solubility of acetylene in human blood and was used to calculate Qc. Helium is used as a

reference point to determine the initial concentration of C_2H_2 in the alveoli after a single breath into the bag. A sample recording is illustrated in figure 1.



Unpublished data from our laboratory verified the reproducibility of the acetylene rebreathe technique. Ten subjects performed cycling exercise at two separate exercise intensities (40% and 60% VO_{2max}) on two separate visits to the laboratory. The order in which the exercise intensity was performed was randomized for each visit with a 2 hour break between each session to allow hemodynamic variables to return to baseline. The Qc was measured every 5 minutes at rest and during exercise. We found the technique to produce highly reproducible Qc values (r^2= 0.88) for the two visits. In addition, the average coefficient of variation for the low and high intensity workload was 7.2% and 8.4%, respectively.

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3. **Triebwasser JH, Johnson RL, Burpo RP, Campbell JC, Reardon WC, and Blomqvist CG**. Noninvasive determination of cardiac output by a modified acetylene rebreathing procedure utilizing mass spectrometer measurements. *Aviat Space Environ Med* 48: 203-209, 1977.