A COMPARISON OF BLOOD FLOW OF THE VASTUS LATERALIS

DURING EXERCISE IN TRAINED AND UNTRAINED CYCLISTS

AS MEASURED BY ^{133}XENON CLEARANCE

by

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ABSTRACT

The purpose of this investigation was to examine muscle blood flow (MBF) in the vastus lateralis of trained and untrained cyclists using the [133]Xenon clearance method. MBF was measured in five trained cyclists (VO2max = 68.8 ± 4.2 ml/kg/min) and five untrained subjects (VO2max = 48.2 ± 4.2 ml/kg/min) at 150 Watts, the anaerobic threshold (AT), and at 100% VO2max. These workloads corresponded to an absolute (150 W), relative (AT) and maximal comparison between groups. [133]Xenon dissolved in saline (3-9 MBq in < 0.2 ml volume), was injected in the vastus lateralis muscle prior to ergometer work. Clearance of the isotope was monitored using a gamma camera positioned adjacent to the injection site (Chung et. al., 1987). MBF calculations are based on the half clearance time (T1/2) during the initial steep portion of the clearance curve, and the blood-muscle partition coefficient value of [133]Xenon (0.7) according to the formula MBF = (In 2/T1/2) * 0.7 * 100 (Clausen and Lassen, 1971). MBF was not significantly different between the two groups at 150 Watts (28.7 ± 3.7 vs. 27.5 ± 7.9 ml/100g/min for trained and untrained groups respectively) or at AT (35.1 ± 8.6 vs. 29.8 ± 2.6 ml/100g/min), but was significantly higher in the trained group at 100% VO2max (35.6 ± 8.1 vs. 27.0 ± 4.3 ml/100g/min). It appears that the high variability in the MBF results may have masked some differences, thereby identifying this limitation in the gamma camera technique. No significant differences were evident across the three exercise conditions in either group, and no significant relationships were detected between MBF and workload (expressed in Watts, ml of oxygen, or % VO2max). The lack of a strong correlation between workload

and MBF indicates that the technique may not accurately track MBF across all exercise intensities. It appears that the gamma camera technique may not be suitable for measuring MBF at high exercise intensities due to the short time course of ^{133}Xenon washout.

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CHAPTER 1

1.1 Introduction

During prolonged exercise, dramatic cardiorespiratory changes are required in order to accommodate the ten to twenty fold increase in total body energy requirements over resting values. These cardiorespiratory changes serve largely to support the increased metabolic needs of active skeletal muscle fibers. Oxygen and nutrients must be provided to the muscle, heat must be removed, and the fluid balance must be maintained. Each of these processes is dependent upon the appropriate delivery of blood flow through the muscle vascular beds.

The measurement of this muscle blood flow (MBF) can serve as a valuable tool in assessing the metabolic state of the working muscle. However, the difficulties involved in measuring MBF during exercise have led to a relatively limited and conflicting view of the hemodynamic response during exercise. The classic method for measuring regional limb blood flow is venous occlusion plethysmography, although there are problems associated with this technique. Motion artifacts make measurements difficult to perform during exercise, and because venous pressure may rise higher than diastolic, MBF will be underestimated due to blood escaping under the collecting cuff (Sullivan et. al., 1987). The ^{133}Xenon clearance method of measuring muscle blood flow, originally proposed by Lassen et. al. in 1964, has been shown to be a valid and reliable measure in the identification of vascular disease, in measuring blood flow in exercising muscle, and in detecting blood flow changes with alterations in intramuscular pressure (Lassen et. al.,

1964; Clausen and Lassen, 1971; Styf, J., 1990; Tonneson and Sejrsen, 1970). Corbally and Brennan (1990) state in their review article of non-invasive measurement of regional blood flow in man, that "clearance techniques offer an attractive alternative to plethysmography and present similar accuracy and reproducibility". The present study used a modified version of the original technique. This modification, introduced by Chung et. al. in 1987, uses a gamma camera positioned adjacent to the exercising limb to record the isotope clearance. The traditional method involves fixing a collimated NaI crystal scintillation detector, or a miniature cadmium-telluride γ-detector, over the injection site. Major advantages of the ^{133}Xenon technique are that it allows for the measurement of blood flow during exercise, which plethysmography does not, it is convenient to administer (no catheters are required) and it can be applied as an indicator of metabolic substrate exchange as it measures blood flow at the capillary level.

Several investigators have found that in endurance trained athletes, MBF is reduced when working at a given submaximal workload (Varnauskas et. al., 1970; Grimby et.al., 1967; Bergman, 1973). This reduced MBF is generally attributed to the increased oxygen extraction capability ($\sim$10%) observed in endurance trained athletes due to local enzymatic adaptations and an increased capillary density (Clausen, 1976). It has also been suggested that peak MBF during maximal aerobic exercise is increased after a period of endurance training. Such an increase seems logical, given the greatly increased cardiac output in endurance trained individuals but experimental evidence is insufficient to conclusively confirm this adaptation (Terjung et. al., 1990). In a study focusing on the hemodynamic and metabolic responses of the human lower limb during exercise, Sullivan

et. al. (1987) have shown that blood flow to the exercising lower limb (measured by thermodilution) correlates well (r = 0.92) with cardiac output. They suggest that the primary determinant of blood flow to the lower limb during exercise appears to be the oxygen requirement of that limb.

1.2 Statement of the Problem

The purpose of this study is to compare the blood flow of the vastus lateralis of trained and untrained cyclists exercising at two relative (anaerobic threshold and VO2max), and one absolute (150 Watts) workload, utilizing the [133]Xenon clearance method of measuring MBF.

1.3 Definitions

Maximal Oxygen Consumption (VO2max) - the maximal rate at which oxygen can be consumed per minute; measures the power or capacity of the aerobic or oxygen system

Anaerobic Threshold (AT) - the point where the aerobic energy response is of insufficient magnitude to supply the tissues energy requirement, resulting in an increase in excess carbon dioxide, and an increased dependence on anaerobic processes

Excess Carbon Dioxide (ExCO2) - non-metabolic CO_2 formed as a result of the hydrogen ions of lactic acid being buffered by bicarbonate in the following reaction:

$$HLa + NaHCO_3 = NaLa + H_2CO_3 = CO_2 + H_2O$$
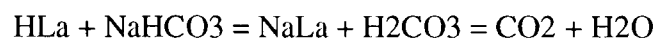
The calculation of ExCO2 will be based on the formula of Volkov et. al. (1975) where:

$$ExCO2 = VCO2 - (RQrest \times VO2)$$

Muscle Blood Flow (MBF) - the blood flow through muscle tissue in a defined region or volume of tissue, expressed in units of ml/100 g/min

Maximal Muscle Blood Flow (maxMBF) - the greatest MBF achieved during whole body exercise (i.e. in this case, cycling)

1.4 Delimitations

This study is delimited by:

1. a sample of 10 males between 20 and 30 years of age

2. the methodology and instruments used in determining MBF, VO2max and the anaerobic threshold

1.5 Limitations

This study is limited by:

1. the individuals metabolic response to the exercise protocols

2. the ^{133}Xenon clearance technique

3. the data collection capabilities of the SensorMedics Vmax 29 Series metabolic measurement system.

1.6 Hypotheses

It is hypothesized that:

a) MBF will be significantly higher in the trained subjects at AT,

b) MBF will be significantly higher at VO2max in the trained subjects,

c) MBF will be significantly lower in the trained subjects at the absolute workload.

The rationale for these hypotheses is as follows:

a) the anaerobic threshold of trained athletes should be at a higher percentage of their VO2max than that of untrained subjects, thus we expect a higher MBF at this higher relative workload (thus the absolute workload will also be higher at AT)

b) the substantial physiological adaptation which occurs with endurance training, including central (cardiac output) and peripheral (capillary density) factors is expected to lead to a greater maximal blood flow

c) given the increased oxygen extraction capabilities of trained muscles, work at the absolute level of 150 Watts will require a higher MBF for the untrained subjects. Since the oxygen requirement will be the same for both groups at this absolute workload, the increased oxygen extraction capabilities of the trained group will allow for a lower MBF.

1.7 Significance of the Study

The majority of methods for measuring blood flow examine flow through an entire limb, such as plethysmography, or they measure the flow through a major vessel, using methods such as ultrasound Doppler, thermodilution, or dye dilution. The clearance of [133]Xenon depends on the capillary blood flow in the muscle, which allows for interpretation of this data as an indicator of metabolic substrate exchange (Corbally and

Brennan, 1990). Some refer to this blood flow as "nutritive flow" (Hickner et. al., 1994). It is well documented that important metabolic changes occur as an individual progresses from an aerobic to an anaerobic state (i.e. the anaerobic threshold). However, MBF, the process which supplies the working muscle with the required nutrients and removes waste products, has not been examined at this critical exercise intensity. Furthermore, disagreement exists as to whether or not maxMBF is higher in endurance trained athletes at maximal work levels as compared to untrained individuals.

This study is also significant in that it will utilize a relatively new, modified (using a gamma camera) version of the standard [133]Xenon clearance technique introduced by Chung et. al. in 1987. This technique is relatively non-invasive (only the injection is invasive), inexpensive, and convenient to administer compared to other MBF measurement techniques such as dye or thermodilution, and ultrasound Doppler.

CHAPTER 2

2.1 Introduction

The main task of circulation is to supply oxygen and nutrients to the tissues and to remove waste products and heat. Skeletal muscle comprises about 40% of total body mass and during strenuous exercise it places the greatest demands on the circulatory system. The 5-8 fold increase in cardiac output from rest to exercising conditions along with the redistribution of blood flow from metabolically inactive regions to active muscle mass can lead to a ~20 fold increase in MBF. The following review will examine our present understanding of MBF during rest and exercise, and the effects of training. The focus of the remainder will be on MBF measurement with the ^{133}Xenon clearance method.

2.2 Muscle Blood Flow

2.2.1 Resting and Submaximal Exercise MBF

Under resting conditions, skeletal muscle receives approximately 15-20% of cardiac output (750-1000 ml/min), or between 2 and 4 ml/100g/min (Rowell, 1986; Elia et. al., 1993). This has been consistently reported in the literature, regardless of the subject population studied or the means of blood flow measurement (Saltin, 1985; Snell et. al., 1987; Chung et. al., 1987; Sullivan et. al., 1987).

With the initiation of dynamic exercise, a series of neural and metabolic events in the working muscle leads to a drop in vascular resistance, subsequently increasing MBF through these muscles. Virtually every metabolite produced by contracting muscle acts as

a vasodilator to some degree. The chemical substances formed by these muscles, in conjunction with a number of other factors, act on the resistance vessels to regulate MBF in proportion to the metabolic demands.

Early studies of hemodynamics during exercise indicated that muscle blood flow increases with exercise intensity in a linear manner (Tonnesen, 1964; Grimby et. al., 1967; Clausen and Lassen, 1971; Pirnay et. al., 1972). Some disagreement existed however, on whether this linear relation existed up to VO2max. Several researchers had described a leveling-off in MBF at 60-70% of VO2max (Tonnesen, 1964; Clausen and Lassen, 1971; Bonde-Petersen et. al., 1975). Clausen and Lassen postulated that the recruitment of additional muscle groups and the increased intramuscular pressure caused by contraction were possible explanations of this phenomenon. Bonde-Petersen et. al., on the other hand suggested that the clearance of [133]Xenon may be diffusion limited at high flow rates. Other studies using [133]Xenon clearance found that MBF does not level off, but may display a decline in its rate of increase after approximately 70% of VO2max (Pirnay et. al., 1972; Grimby et. al., 1967). Saltin (1985) found that leg BF increased in a linear fashion with increasing work rate with no tendency to decline at maximal work rates while performing knee extensions (i.e. not whole body exercise) at a rate of 60 contractions/min. Similar results were reported by Radegran (1997) who used ultrasound Doppler and thermodilution to measure BF. Blood flow increased linearly ($r = 0.998$, p ,< 0.001) with exercise intensity, from 0.31 l/min under resting conditions to 7.22 l/min at 70 Watts during one-legged dynamic knee extensor exercise. A strong positive correlation ($r =$

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0.997, p < 0.001) was found between the femoral artery Doppler BF and simultaneously determined thermodilution venous outflow measurements.

Sullivan et. al. (1987) measured leg blood flow (sampled for lactate, catecholamines, and oxygen content), cardiac output, and oxygen consumption in 12 healthy individuals during various stages of cycle ergometry using a thermodilution technique. Covariance analysis suggested that leg oxygen consumption was the primary determinant of leg blood flow and vascular resistance during exercise. Leg blood flow increased in a near linear manner from 0.50 l/min under resting conditions, to 6.75 l/min at maximal exercise intensity.

Technological difficulties in measuring MBF at higher exercise intensities has led to some confusion in the literature, but it is now agreed that MBF does not level off at submaximal exercise intensities.

2.2.2 Maximal Exercise MBF

During exercise utilizing a large muscle mass, it is generally accepted that maximal MBF ranges from 60-120 ml/100g/min (Saltin, 1988; Armstrong, 1988). Sample calculations for a 75 kg subject with 30 kg of muscle mass and a cardiac output of 22 l/min at VO2max, show that if 85% of cardiac output goes to the working muscle, the total MBF would be about 18 l/min, or 125 ml/100g/min if half of the muscle mass (15 kg) is uniformly engaged. Jorfeldt and Wahren (1971) estimated total limb MBF to be 52 ml/100g/min with dye dilution during cycling. Grimby et. al. (1967) found vastus lateralis BF to be 49 ml/100g/min during cycling at 99% of VO2max in a group of 15 trained and

untrained subjects. Pirnay et. al. (1972) found a similar value of 43.2 ml/100g/min in four subjects cycling under maximal conditions. [133]Xenon was used in both of these studies. Cerretelli (1986) suggests that a correction factor of 2 should be applied to [133]Xenon MBF values. The use of such a correction factor brings [133]Xenon MBF values into the same range as estimated MBF values from limb blood flow measurements using techniques such as thermodilution or ultrasound Doppler. Corrected values also correspond better to MBF values calculated from Fick's equation (Cerretelli, 1984, 1986). Sullivan et. al. (1987) measured a peak blood flow of 6.75 l/min during maximal bicycle ergometer exercise using thermodilution. Several other authors have found maximal leg blood flow values in the 5 - 7 l/min range during one-legged dynamic knee extensor exercise (Radegran, 1997; Saltin, 1985).

Saltin (1985) has demonstrated that peak values for MBF may be 2 to 3 times greater when a small muscle mass is active as compared to whole body exercise such as cycling or running. Due to the extremely short mean transit time (MTT) accompanying high flow rates, oxygen extraction by the tissues is much lower, indicating that the flow is excessive. A cardiac output of 50-60 l/min would be required to accommodate such high flow rates during whole body exercise. When exercising with a larger muscle mass the needs of the contracting skeletal muscle surpasses the pump capacity of the heart and sympathetic vasoconstrictor activity must override any vasodilatory factors in order to maintain blood pressure (Secher, 1977; Saltin, 1988; Rowell, 1988).

During the transition from submaximal to maximal exercise, a preferential redistribution of cardiac output occurs directing flow to the active muscles from "non-

exercising" areas (Rowell, 1974; Jorfeldt and Wahren, 1971). Sullivan (1987) found that blood flow to the non-lower limb areas fell significantly from 8.35 ± 1.78 l/min at submaximal exercise (mean VO2 of 1.75 ± 0.21l/min) to 6.3 ± 2.48 l/min at maximal exercise ($P < 0.01$). This vasoconstriction of non-exercising areas is probably evoked by increasing sympathetic nervous system activity and the release of norepinephrine. At high exercise intensities the redistribution of cardiac output is critical in order to maintain adequate MBF, given that the increment in cardiac output decreases between submaximal and maximal exercise.

2.2.3 Control of Blood Flow

The flow of blood through the resistance vessels is directly related to the pressure gradient driving the flow and inversely related to the resistance to flow. Arterial and venous pressures are generally constant, thus the resistance to flow is the controlling factor. This resistance is controlled by variations in the contractile activity of the vascular smooth muscle (VSM) of the blood vessels. The primary factors determining this contractile activity can be divided into three general categories: neural-hormonal, metabolic, and myogenic.

Research indicates that muscle vascular resistance can be influenced by neural-hormonal factors via carotid and aortic baroreceptors, cardiopulmonary receptors, chemoreceptors and by the stimulation of somatic afferents within the skeletal muscles (Laughlin et. al., 1987). The site of these reflex effects appears to be the vascular adrenergic receptors in the resistance vessels which are affected by sympathoadrenal

influences. Such influences are primarily responsible for controlling blood flow at rest and are not believed to be the primary determinant of blood flow during exercise. Rather, exercise hyperemia is generally considered to be a local metabolic phenomenon, with the sympathoadrenal influences being superimposed upon this local tone (Skinner, 1975; Clausen, 1976; Shepherd, 1983; Laughlin et. al., 1987).

Measurement of limb vascular resistance at the onset of exercise produces a biphasic curve displaying a fast initial drop followed by a more gradual reduction (Sullivan et. al., 1987). The initial rapid increase in MBF (or drop in vascular resistance) which occurs with the initiation of dynamic exercise is in response to a neural "on signal" (Laughlin et. al., 1987). This "on response" increases blood flow dramatically and appears to be linked to the frequency of muscle contraction rather than the intensity of exercise. Sheriff et. al. (1993) found that doubling the contraction frequency by increasing treadmill speed also doubled the initial rise in vascular conductance, whereas increasing the treadmill grade from 0% to 10% led to no change in the initial rise. After 15-30 seconds MBF appears to be more closely related to metabolic rate. This matching of blood flow to metabolic demands is most likely achieved through the effects of various metabolic and neurohormaonal factors (such as a decreased blood or tissue PO2, decreased pH, increased PCO2, osmolarity, epinephrine, adenosine, and various prostaglandins) which are produced during exercise.

Current thought in the field indicates that MBF during exercise depends primarily on metabolic vasodilation and the effects of physical forces produced by the rhythmic contraction and relaxation of the working muscles.

2.2.4 Effects of Training on MBF

In 1967 Grimby et. al. performed a study examining MBF during submaximal and maximal exercise in untrained and well-trained subjects. It was found that trained and untrained subjects had identical MBF at equal relative workloads and that MBF increased gradually with increasing work levels but displayed a declining rate in flow increase when approaching maximal work levels. Thus at a given absolute workload MBF is lower in trained subjects than untrained.

Similar results were obtained by Varnauskas et. al. (1970) in measuring the effects of training on MBF. Six weeks of physical training decreased MBF by 31%, or from 32.7 to 22.6 ml/100g/min. These MBF measurements, both pre-training and post-training, were performed at 60% of VO2max measured pre-training. Given the 30% increase in VO2max recorded post-training, this workload represented only 45% of VO2max post-training. It was concluded that an increased oxygen extraction and a more adequate distribution of capillary blood flow were responsible for the reduced post-training MBF. Bergman et. al. (1973) also performed a training study which resulted in a 23% reduction in MBF. MBF was measured at 78% of VO2max pre-training and 68% post-training, given the 18% increase in VO2max as a result of the 6 week training program. Subjects in this study were middle aged (54-55 yrs) men and it was concluded that the adaptive training response described by Varnauskas et. al. (1970) applies to this subject population as well.

Numerous studies have demonstrated an increased capillarization in response to training (Holloszy, 1973; Gollnick et. al., 1982). An increased capillary density not only reduces diffusion distances (along with an increased mitochondrial density) but also increases total capillary volume and cross sectional area. This increase in volume reduces red blood cell velocity at a given capillary flow rate, thereby increasing the mean transit time (MTT) available for oxygen exchange. Saltin (1985) states that, "the primary importance of enlargement of the capillary bed with endurance training is not to accommodate flow but to maintain or elongate MTT".

According to Terjung et. al. (1990), an increased MBF at VO2max should be expected after training due to a greater capacity of the vascular circuit, and/or a greater dilation of the resistance vessels. Such an increase has been demonstrated by some researchers (Leinonen et. al., 1978; Klausen et. al., 1982), while others have failed to show any change. Grimby et. al. (1967), found no differences in MBF at 100% of VO2max in trained and untrained subjects despite a ~5 l/min difference in maximal cardiac output. They attributed this finding to the larger muscle mass employed by the trained subjects, indicating that the higher cardiac output was supplying an increased amount of muscle thus not altering flow per unit tissue. Leinonen et. al. (1978) on the other hand, detected a significantly higher MBF in athletes as compared to control subjects measured during reactive hyperemia from vigorous dorsiplantar flexions of the ankle to exhaustion (86.7 ml/100g/min vs. 62.9 ml/100g/min). Capillary permeability was also measured in this study and it was found that this value was 48% greater in athletes. It was concluded

that an increase in the total capillary bed surface area was responsible for the higher permeability observed in athletes.

The mechanisms for vascular adaptation induced by chronic exercise training can be divided into two major categories: structural adaptation and adaptations in vascular control. Structural adaptation involves vascular remodeling and growth (increased length and cross sectional area of existing vessels) as well as angiogenesis (increased numbers of microvessels per gram of muscle). Changes in vascular control can be the result of (1) altered neurohormonal control of the vascular bed; (2) altered local control via changes in metabolic control systems, altered myogenic responses to mechanical stimuli, intrinsic changes in vascular smooth muscle cells; or (3) the effects of structural changes on the distribution of resistance throughout the microcirculation (Shepherd, 1983).

2.3 MBF Measurement

The measurement of MBF during exercise has proven to be very difficult in vivo. The concept of determining MBF through measuring the clearance of a tracer from a tissue was originally developed by Kety in 1949. The only available method for MBF measurement before this was plethysmography, a technique first introduced in 1732 by Swammerdam. Plethysmographic recordings are generally inapplicable to exercise conditions as the pressure in veins may rise higher than the diastolic pressure, thus underestimating blood flow as blood escapes under the collecting cuff (Tönnesen, 1964). A tracer may be either radioactive or a dye and its' clearance must be principally limited by MBF. The ^{133}Xenon clearance method is based on the assumption that the equilibration of

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this inert gas, between tissue and blood, is so rapid that its disappearance from the muscle is limited by blood flow only. Radioactive tracers are gamma emitters which allow for non-invasive external detection of the rate of tracer washout from the tissue. The decrease of local radioactivity with time, assumed to be a perfusion dependent process, is considered indicative of the effective MBF. The isotope used by Kety was ^{24}Na, which was later changed to ^{133}Xenon by Lassen et. al. (1964), as it is a lipophilic inert gas and the diffusion equilibrium is better maintained than with hydrophilic Na$^+$, which does not readily cross cellular membranes. ^{133}Xenon passes freely across cell membranes and its movement depends on solubility and diffusion gradients. As it is a soft gamma-emitting (81 keV) isotope which is eliminated from the lungs during its first passage through the pulmonary circulation (blood to air partition coefficient of 10:1), patient safety is assured during repeated examinations (physical half-life = 5.27 days, physiological half-life < 1 hour).

Lassen et. al. (1964) used this technique to measure MBF (reactive hyperaemia) in healthy subjects and patients with occlusive arterial disease of the legs. At rest MBF was not significantly different in the two groups, but after work induced ischemia, maximal MBF averaged 17 ml/100g/min in patients with vascular disease and was significantly higher in healthy subjects above 50 years of age at a value of 55 ml/100g/min. Repeated MBF tests were carried out on different days and the coefficient of variation was found to be 13% for flow values above 30 ml/100g/min and 20% for flow values below 30 ml/100g/min, based on a total of 49 measurements. Other researchers have also found the coefficient of variation to be between 15 and 20 % (Clausen and Lassen, 1971; Grimby

et. al., 1967; Pirnay et. al., 1972). MBF calculations were based on the Fick principle and the assumption of maintenance of complete or almost complete diffusion equilibrium, $C_{muscle} = \lambda \times C_{blood}$ (where C_{muscle} is the amount of ^{133}Xenon per gram of muscle, C_{blood} is the amount of ^{133}Xenon per ml of blood, and λ is the muscle/blood partition coefficient). This muscle/blood partition coefficient has been determined to be 0.70 for blood with a haematocrit of about 40 (Conn, 1961).

The first study using this technique to measure MBF during exercise was performed by Tönnesen in 1964. Gastrocnemius blood flow was examined during 6 minutes of plantar-flexion through 45° of movement. Results indicated that MBF rises linearly with increasing work intensities up to about half of maximal work capacity (corresponding to a MBF of about 40 ml/100g/min), after which no further increases were evident. A comparison of these results to those of Lassen et. al. (1964) showed that the maximal hyperemia flow was 30% greater than that measured during exercise. Tönnesen suggested that the pressure developed in the muscle during contractions led to this discrepancy.

In contrast to the leveling off in MBF observed by Tönnesen (1964), Grimby et. al. (1967) found that MBF increased up to VO2max, with a tendency towards a smaller rate of increase nearing maximal levels. The muscle studied in this instance was the vastus lateralis during cycling, with MBF measurements being performed at 24, 48, 75 and 99% of VO2max in trained and untrained subjects. Mean values for MBF were 15, 28, 43, and 49 ml/100g/min. Similar results were reported by Pirnay et. al. (1972), who measured MBF during cycling between 50 and 300 Watts (at 50 Watt intervals). MBF increased

from 13.3 ml/100g/min to 53.1 ml/100g/min, displaying a slower rate of increase as exercise neared maximum. When MBF was occluded in one leg during maximal exercise it was found that MBF in the opposite leg exceeded the value obtained when exercising with both legs.

In 1970, Tönnesen and Sejrsen examined the validity of the [133]Xenon clearance technique, as well as the effect of injection trauma on MBF results. Direct measurement of venous output was compared to the [133]Xenon clearance technique in the isolated cat gastrocnemius. A significant correlation (r = 0.85) was found between the directly measured venous outflow and values calculated from [133]Xenon clearance over a wide range of conditions. Experimental support was also found for the basic assumption of the clearance method, that [133]Xenon is able to maintain diffusion equilibrium between tissue and blood during alterations in blood flow. Attempting to minimize injection trauma by using depots of less than 0.1 ml led to non-significant correlation coefficients between the two MBF measurement methods. However, the trauma of injection could be reduced by allowing for an appropriate waiting period (5 to 7 minutes, or as soon as a steady slow clearance rate was obtained) between the injection and the onset of exercise.

Clausen and Lassen (1971) also examined the [133]Xenon technique during exercise in an attempt to evaluate the conflicting findings of Tonnesen (1964) and Grimby et. al. (1967). This study evaluates the experimental technique as well as the relation between blood flow and work load. Attempts to minimize scatter of the MBF values through variations in injection depth and the site of injection were unsuccessful. It was concluded that the muscle tissue is sufficiently inhomogenous, at least after [133]Xenon injections, to

result in a considerable scatter (15-20%) of the MBF values. Steady state values for MBF were attained less than 1 minute after the onset of exercise or change in the workload. When exercising between 20 and 70% of VO2max, MBF was proportional to the work intensity, averaging 21 and 51 ml/100g/min respectively. No further increases in MBF were seen after 70% of VO2max, and supramaximal work led to a reduction in MBF. Investigations into the possibility that inherent limitations in the [133]Xenon method led to this leveling off in MBF at submaximal exercise intensities were undertaken and it was concluded that such limitations do not exist.

Cerretelli et. al. (1984) compared blood flow values obtained by the [133]Xenon method to direct venous outflow and microsphere trapping flow determinations in isolated perfused dog gastrocnemius at rest and during graded stimulation, and in the gastrocnemius, vastus lateralis, and triceps of intact dogs at rest and while running on a treadmill at varied speeds up to maximum. Directly measured venous outflow was found to be almost identical to MBF measured by microsphere trapping at rest and at all levels of stimulation in 29 measurements performed in 11 isolated muscles. [133]Xenon clearance yielded values which were on average only 57% of those obtained by the other two methods. Measurements performed on intact dogs displayed similar results, with [133]Xenon clearance values averaging 49% of the values obtained by microsphere trapping, independent of blood flow. It was concluded that the [133]Xenon clearance method underestimates MBF by 50% and that results should be given a qualitative rather than quantitative significance. They also suggest the possibility of using a correction factor to

estimate true MBF from ^{133}Xenon clearance results, noting that "corrected" ^{133}Xenon values are compatible with MBF values calculated from Fick's equation.

An alternative method to the miniature probe system for detection of ^{133}Xenon clearance was developed by Chung et. al. (1987). The miniature cadmium-telluride γ-detector system conventionally used is not available in most nuclear medicine departments. It was therefore proposed that a gamma camera and on-line computer be used to measure ^{133}Xenon clearance. MBF was calculated to be 2.15 ml/100g/min under resting conditions and 24.5 ml/100g/min while cycling at 25 Watts less than the previously determined maximal load. It was stated that these values are in accordance with normal values in the literature, such as those of Lassen et. al. (1964) and Clausen and Lassen (1971), thus this system can be used instead of a detector probe system for the measurement of MBF during rest and exercise.

CHAPTER 3

3.1 Subjects

Ten male subjects (five untrained and five highly trained cyclists) volunteered to take part in this study. Prior to testing, subjects received a detailed explanation of the experimental procedures, which were approved by the University of British Columbia Ethics Committee, and read and signed an informed consent. Subjects were asked to refrain from heavy exercise 24 hours prior to testing and to be 3 hours postabsorptive.

3.2 Testing Procedures

Initial testing took place in the J. M. Buchanan Exercise Science Lab at the University of British Columbia. Each subject completed a graded cycle ergometer test to determine their VO2max and AT. The results of this testing were used to determine individual workloads for each subject which elicited 100% of VO2max and AT. Subsequent testing was performed in the Division of Nuclear Medicine (VHHSC, UBC Site) where on three separate days, MBF measurements were made at the pre-determined workloads. During all testing sessions heart rate was measured using a Polar Accurex® heart rate monitor (Model #1900400).

3.3 Testing Protocols

3.3.1 Maximal Oxygen Consumption (VO2max)/ Anaerobic Threshold (AT) Determinations

All tests were preceded by a warm-up consisting of an explanation of the testing procedures followed by a physiological warm-up against a light resistance with all equipment in place. Subjects were instructed to maintain a comfortable cadence of 80-90 rpm, while cycling on an electronically braked SensorMedics 800 bicycle ergometer. The protocol began at an initial workload of 50 Watts and progressively (ramp) increased at a rate of 30 Watts per minute until volitional fatigue (the inability to maintain the prescribed cadence or the subjects own termination of the test) was reached. Attainment of VO2max was according to the following criteria:

1) The oxygen consumption ceases to increase linearly with rising workload and approaches a plateau or drops slightly, the last two values agreeing within ± 2 ml/kg/min.

2) Heart rate should be close to the age predicted maximum.

3) The respiratory exchange ratio (RER) is greater than 1.10.

Respiratory and gas exchange variables (VO2, VCO2, RER, ExCO2) were collected and analyzed using a SensorMedics Vmax 29 Series metabolic measurement system.

The anaerobic threshold was determined at the point where a disproportionate increase in the ExCO2 elimination curve over time occurs (Frangolias and Rhodes, 1995).

3.3.2 Muscle Blood Flow Testing

Prior to each test, [133]Xenon in saline was prepared by injecting ~4 ml (to the maximum volume of the vile) of sterile, normal saline into a vial of commercial [133]Xenon containing 370 Mbq. This was stored in a refrigerator for 24-36 hours.

The ergometer bicycle (Monark, Model 868) was positioned next to a gamma camera (Siemens ZLC3700 Orbiter equipped with a high sensitivity collimator) so that the site of injection remained within the effective field of the camera during cycling and the thigh remained in close contact with the camera collimator. Subjects warmed up briefly against a light resistance while the testing procedure was being explained. One to two minutes after the warm-up, an injection of [133]Xenon (3-9 Mbq in < 0.2 ml volume) was made in the v. lateralis 5-10 cm proximal to the lateral epicondyle of the femur. The injection was made with a 27-gauge, petcock equipped needle at a depth of 1.5-2.5 cm. The exact injection site was noted for each individual to ensure consistency between tests. After a two minute period of rest (to minimize the effects of injection trauma) subjects begin to exercise at the predetermined workload, while the activity of [133]Xenon was recorded by the gamma camera ICON A™ data acquisition system at 6 second intervals. Exercise bouts lasted from 1.5-5 minutes depending on the exercise intensity. Tests at 100% of VO2max lasted for a minimum of 1.5 minutes, while tests at AT and 150 Watts were 5 minutes long.

A region of interest was drawn around the curvilinear image produced by the [133]Xenon activity (see Figure 1), from which count numbers were obtained for each 6 second interval, allowing for the half disappearance rate of [133]Xenon to be calculated (see Figure2). This half time was calculated from clearance data during the initial 90 seconds of each test. It was during this time period that the rapid portion of the decay curve occurred which represents [133]Xenon washout from true muscular tissue (Cerretelli, 1984). MBF calculations were based on the half clearance time ($T^1/_2$) of [133]Xenon, and the blood-

^{133}Xenon, and the blood-muscle partition co-efficient (λ) value of ^{133}Xenon ($\lambda = 0.7$, Conn, 1961), according to the formula MBF = (In 2/ T^1/$_2$) $\times$ λ $\times$ 100 (Clausen and Lassen, 1971).

FIGURE 1: ^{133}XENON ACTIVITY RECORDED DURING EXERCISE (6 SEC. INTERVALS)
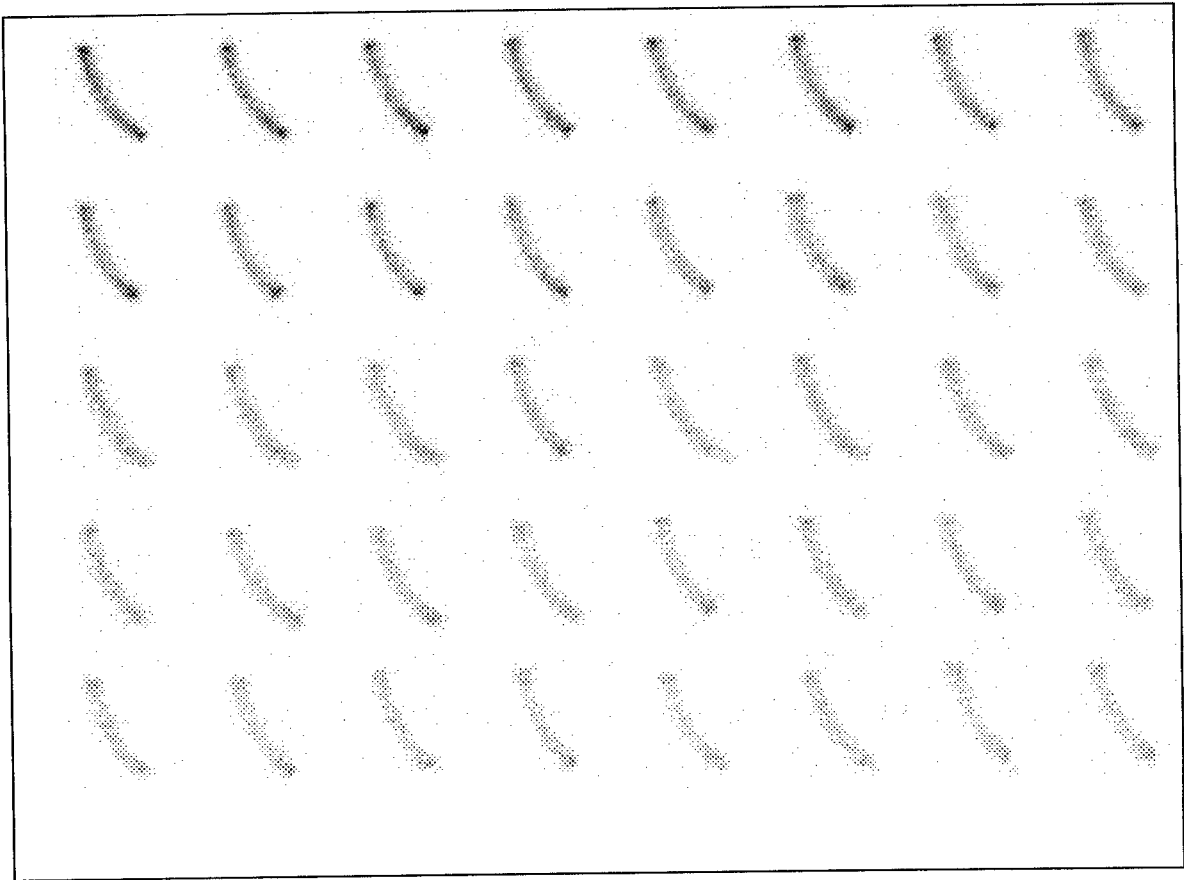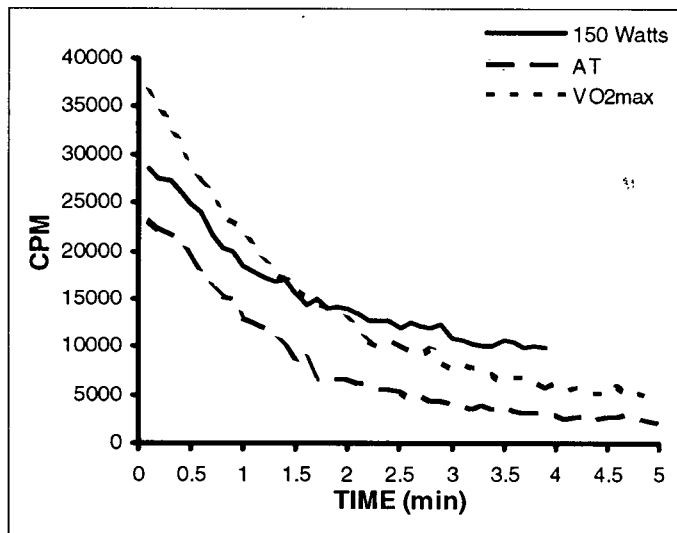

Figure 1 displays a recording of the isotope washout during exercise. Each picture represents the amount of activity present during 6 seconds of exercise. This activity, measured in counts per minute (cpm), is determined for each 6 second interval and a clearance curve is then generated (Figure 2).

FIGURE 2: ^{133}XENON CLEARANCE CURVE

3.4 Data Analysis

Data analysis was performed as follows;

1. Group MBF means were compared at each work intensity using an independent t-test.

T150 vs. UT150

TAT vs. UTAT

Tmax vs. UTmax

2. Within each group, MBF was examined for significant differences between the three exercise conditions using simple ANOVA. If necessary, a Newman-Keuls post-hoc comparison was performed.

T150 vs. TAT vs. Tmax

UT150 vs. UTAT vs. UTmax

3. MBF was correlated with workload (expressed as % VO2max and in Watts), and with VO2 in ml/kg/min for each group using the Pearson product moment correlation (*r*).

The level of significance was set at $p < 0.05$ for all statistical procedures performed.

CHAPTER 4

4.1 Subjects

Ten male cyclists, five untrained and five highly trained, completed this study. Subjects physical characteristics are presented in Table 1.

TABLE 1: CHARACTERISTICS OF SUBJECTS

CHARACTERISTIC	X ± SD TRAINED (N=5)	X ± SD UNTRAINED (N=5)
AGE (YRS)	25.6 ± 1.34	24.8 ± 2.86
HEIGHT (CM)	179 ± 2.24	177.2 ± 6.3
MASS (KG)	72.26 ± 2.03	75.08 ± 9.72
VO2MAX (ML/KG/MIN, L/MIN)	68.78 ± 4.17; 4.97 ± 0.23	48.24 ± 4.18; 3.6 ± 0.46
AT (% VO2MAX)	67.40 ± 5.55	70.8 ± 8.70

4.2 Muscle Blood Flow

The muscle blood flow (MBF) results obtained are presented in Table 2 and Figure 3. In addition to MBF results, Table 2 also displays the percentage of VO2max each workload represented for each group as well as the workload in Watts.

Mean values (± SD) for MBF in the untrained group were 27.5 ± 7.9, 29.8 ± 2.6, and 27.0 ± 4.3 ml/100g/min at workloads of 150, 192, and 325 Watts, or 52, 72, and 100% of VO2max. These workloads corresponded to to an absolute (150 W), relative

(AT), and maximum comparison between groups. ANOVA revealed no significant differences in MBF values between the three exercise conditions (p > 0.05). Results of the trained group were 28.7 ± 3.7, 35.1 ± 8.6, and 35.6 ± 8.1 ml/100g/min at workloads of 150, 285, and 444 Watts, or 41, 67, and 100% of VO2max. No significant differences were found between conditions (p > 0.05).

Statistical analysis revealed no significant differences in MBF between the untrained and trained group at the 150 Watt intensity or the AT workload (p > 0.05). MBF was significantly higher in the trained group under maximal exercise conditions (35.6 vs. 27.0 ml/100g/min, p < 0.05).

TABLE 2: MUSCLE BLOOD FLOW DATA (X ± SD)

	MBF ML/100G/MIN	% VO2MAX	WORKLOAD IN WATTS
150 WATTS			
TRAINED	28.7 ± 3.7	41 ± 3.9%	150
UNTRAINED	27.5 ± 7.9	52 ± 7.6%	150
ANAEROBIC THRESHOLD (AT)			
TRAINED	35.1 ± 8.6	67 ± 5.6%	285 ± 31
UNTRAINED	29.8 ± 2.6	72 ± 9.9%	192 ± 34
MAX (VO2MAX)			
TRAINED	35.6 ± 8.1 *	100	444 ± 21
UNTRAINED	27.0 ± 4.3 *	100	325 ± 39

* Significantly different at $P < 0.05$

FIGURE 3: MUSCLE BLOOD FLOW
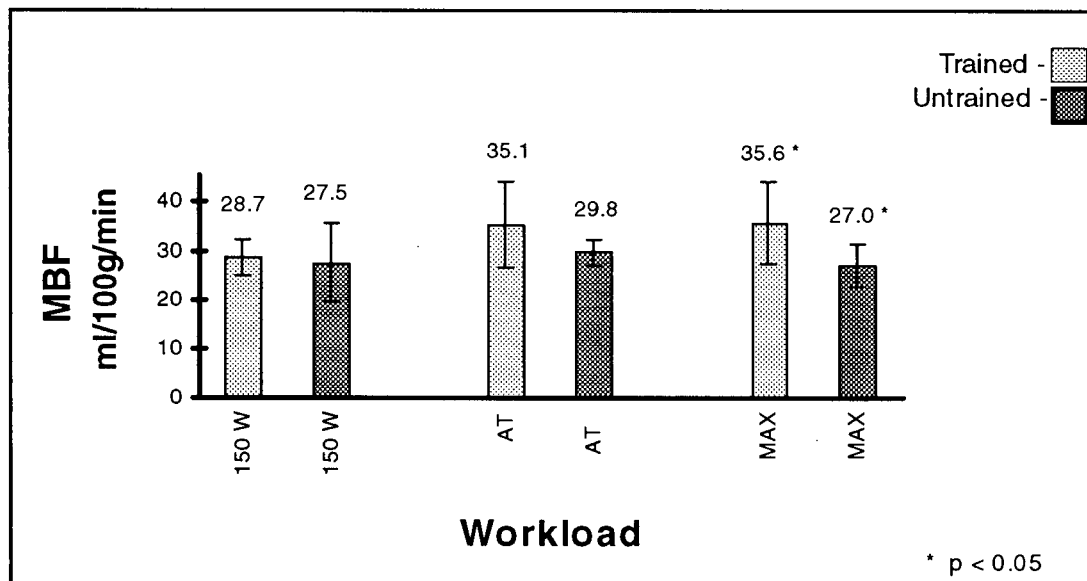
The relationships (Pearson product moment r's) between MBF and workload (expressed as; Watts, % VO2max, and ml of oxygen) are shown in Table 3. The trained group displayed moderate correlations (r = 0.4, 0.39, and 0.43) between MBF and workload (expressed in Watts, % of VO2max, and in ml of oxygen, respectively). Correlations in the untrained group were much lower (r = -0.14, 0.06, and -0.02).

TABLE 3: MBF CORRELATIONS (r)

	WORKLOAD IN WATTS	WORKLOAD AS % VO2MAX	WORKLOAD IN ML O2
TRAINED	r = 0.4	r = 0.39	r = 0.43
UNTRAINED	r = -0.14	r = -0.02	r = 0.06

CHAPTER 5

5.1 Discussion

5.1.1 Introduction

The measurement of muscle blood flow (MBF) during whole body exercise in humans has proven to be greatly limited by technical difficulties and/or open to measurement limitations. Due to the complexity of such experiments, many researchers have used new innovative methods to measure MBF. The use of radiolabeled microspheres in dogs (Cerretelli, 1986), and the introduction of the one-legged knee extensor model utilizing the thermodilution technique (Saltin, 1985) or ultrasound Doppler (Radegran, 1997) are examples of newer techniques. The ^{133}Xenon clearance method non-invasively measures MBF within a given region or volume of muscle tissue (at the capillary level) during exercise (Elia, 1993), which is assumed to be indicative of the metabolic state of the working muscle. Other MBF measurement techniques used in exercise studies, such as ultrasound Doppler, thermodilution, and dye-dilution measure total limb blood flow through a major vessel, and are not as convenient to administer as the ^{133}Xenon technique.

5.1.2 Muscle Blood Flow

Muscle blood flow (MBF) values at the absolute workload of 150 Watts were hypothesized to be higher in the untrained group, who were working at a higher percentage of their VO2max (52 vs. 41%), however no significant difference was found.

Values of 28.7 ± 3.7 ml/100g/min for the trained group and 27.5 ± 7.9 ml/100g/min for the untrained group are similar to the results of other investigators. At 50% of VO2max, Grimby et. al. (1967) found MBF to equal 28 ml/100g/min, while Pirnay et. al. (1972) measured MBF to be 24.3 ml/100g/min at 150 Watts. Varnauskas et. al. (1970) reported MBF to be 33 ml/100g/min at 60% of VO2max, which dropped to 22 ml/100g/min at 45% of VO2max post-training.

The results of training studies indicate that MBF is reduced (18-23%) when measured at the same absolute workload post-training (Bergman et. al., 1973; Varnauskas et. al., 1970). Similarly, comparisons between trained and untrained subjects have shown that at equal relative workloads, trained and untrained subjects have identical MBF's (Grimby et. al., 1967). Presumably, the increased oxygen extraction (a-vO2 difference) capabilities observed in trained individuals allows for a reduced MBF to supply sufficient oxygen to the working tissues (Terjung et. al., 1990). The present study does not confirm these findings as MBF was not lower in the trained subjects at the absolute workload of 150 Watts. A difference of approximately 5 ml/100g/min was expected but high variability in the results (the standard deviation in the untrained group was 7.9 ml/100g/min) may have made such a difference undetectable.

The MBF measurements at the anaerobic threshold (AT) were not significantly different from those at the 150 Watt workload for either group despite the fact that the AT workload was on average 42 Watts greater in the untrained group and 135 Watts greater in the trained group. Average values for MBF did increase from 28.7 to 35.1 ml/100g/min in trained subjects, and from 27.5 to 29.8 ml/100g/min in the untrained, but

high variability in the results may have masked possible differences. Pirnay et. al. (1972) reported a MBF of 43.2 ± 10.1 ml/100g/min at 300 Watts, somewhat higher than the MBF of 35.1 ml/100g/min recorded in the trained group at 285 Watts (AT) in the present study. At 200 Watts, comparable to the untrained AT workload of 192 Watts, Pirnay et. al. (1972) found MBF to be 30.7 ± 3.2 ml/100g/min, very close to our measured value of 29.8 ± 2.6 ml/100g/min. Comparison between the two groups at this workload revealed that the MBF values of 35.1 ± 8.6 ml/100g/min for the trained group and 29.8 ± 2.6 ml/100g/min for the untrained group are not significantly different from each other.

Under maximal exercise conditions, the MBF values obtained were not significantly different from those at either of the submaximal workloads in either group. In the untrained group the average measured MBF of 27.0 ± 4.3 ml/100g/min was actually lower than at the two submaximal workloads but still not significantly different. MBF in the trained group was 35.6 ± 8.1 ml/100g/min, significantly higher than in the untrained group. This is in agreement with the hypothesis that MBF would be higher in the trained group at the maximal workload but one must question the validity of this finding given the low MBF found in the untrained group at 100% of VO2max. The low MBF value observed in the untrained group may be due to a slower response in untrained individuals at the onset of exercise (Shoemaker et. al., 1996).

Chung et. al. (1987), who introduced the gamma camera modification, found MBF at 25 Watts below the predicted VO2max to equal 24.5 ± 9.95 ml/100g/min. This value is quite close to our measured maximal MBF's of 35.6 and 27.0 ml/100g/min at 100% of VO2max. However, in comparison to the results of other researchers, these maximal

MBF values appear to be far too low. Grimby et. al. (1967) reported an average maximal MBF of 49.0 ml/100g/min. Similar values have been found in other studies using the [133]Xenon technique; 54.9 ml/100g/min (Lassen et.al., 1964), 43.2 ml/100g/min at 300 Watts (Pirnay et. al., 1972), and 51 ml/100g/min (Clausen et. al., 1971). Data from thermodilution techniques (Sullivan et. al., 1987; Saltin, 1985) and from ultrasound Doppler (Radegran, 1997) indicates that maximal MBF to the lower limb may be as high as 5 - 7 l/min. The mass of the quadriceps muscles has been measured by several researchers as being in the 2.5 to 3.5 kg range (Saltin, 1985; Radegran et. al., 1998). Therefore, if half of the limb blood flow were to perfuse the quadriceps muscle, a MBF of about 120 ml/100g/min would be expected.

5.1.3 Methodological Concerns

Clearance curves obtained in the present study display the characteristic shape (see Figure 2) as described by Cerretelli et. al. (1984). Such curves are characterized by 1) an initial very fast component (accounting for about 10 % of the initial counting rate decrease); 2) by a fast monoexponential segment covering the range 90 % to about 20-30 % of the initial counting rate; and 3) by a slow component. The amount of activity injected was determined in order to ensure that sufficiently high initial count numbers were obtained to allow for monitoring of isotope clearance. This was done by measuring the syringe activity pre- and post- injection and resulted in an average of 5.5 MBq being injected in less than 0.2 ml volume.

The initial count rates obtained using a gamma camera were substantially lower than those obtained in other [133]Xenon studies which used portable detectors taped over the injection site. This is most likely a result of the greater distance between the injection depot and the detection device. Initial count rates in the present study ranged from 20000 cpm to 55000 cpm with the average being approximately 32000 cpm. Specific count numbers are rarely mentioned in other [133]Xenon studies, but sample figures from these studies display initial counts of 50000-100000 cpm. This should not effect the shape of the curve but it does reduce the length of time that is useful for MBF measurement. This is the steep portion of the curve which occurs in the first two to three minutes (see Figure 2), before the slow component of the washout curve is reached. Some researchers use an analysis in which the slow component is peeled off. This is based on the reasonable assumption that the slow component represents [133]Xenon washout from non-muscular tissue such as fat or connective tissue. According to Cerretelli, when this slow component is not peeled off calculated MBF values are about 35 % lower than MBF calculated from the fast component only. Because of the short time course of Xenon washout measured with a gamma camera, we were unable to calculate this slow component, hence the MBF values reported may underestimate true MBF by 35%. This however would effect the results equally at all exercise intensities, thus not altering the qualitative nature of the data (Cerretelli, 1984; 1986).

The Xenon method evaluates blood flow in a rather small volume and inhomogeneous perfusion of skeletal muscle has been reported (Vicini et. al., 1997; Cerretelli et. al., 1986; Terjung et. al., 1990). This may account for some of the variation

seen with this method. The high variability of MBF values in the present study must be taken into consideration when interpreting the results. Variability of MBF values measured using the [133]Xenon clearance technique has been consistently reported as being in the range of 10-20% (Clausen et. al., 1971; Pirnay et. al., 1972). These values were determined for the traditional method which involves the use of lightweight scintillation detectors to measure Xenon clearance whereas the present study utilizes a gamma camera to monitor isotope clearance as described by Chung et. al. (1987). Examination of the results in the Chung et. al. (1987) study reveal that variability was also very high. MBF measured at rest was 2.15 ± 1.33 ml/100g/min and at the submaximal workload of 25 Watts less than VO2max, MBF was 24.5 ± 9.95 ml/100g/min resulting in coefficient of variation values of 62% and 41% respectively. In the present study these values were much lower, but still very high, ranging from 9% to 29%. Therefore, it is likely that small differences in MBF would not be detected using this method unless a very large number of subjects were tested.

The MBF values obtained at both the AT workload and VO2max appear to be rather low. This is especially apparent when looking at the trained subjects MBF under the three exercise conditions. In this group MBF was not significantly different between the three exercise conditions (28.7 vs 35.1 vs 35.6 ml/100g/min) despite the fact that the workload increased from 150 Watts to 285 Watts at AT, to 444 Watts under maximal conditions. MBF under maximal exercise conditions has been reported by other authors as being in the 45-60 ml/100g/min range using the [133]Xenon method (Grimby et. al., 1967; Clausen et. al., 1971; Pirnay et. al., 1972). More evidence comes from sample

calculations which indicate that maximal MBF during whole body exercise should be at least 75 ml/100g/min. This is based on a 75 kg subject with about 30 kg of skeletal muscle (40 % body mass) which is being supplied by a cardiac output of 25 l/min, 85% of which is directed to skeletal muscle. The active muscles should receive a flow of at least 75 ml/100g/min because it is unlikely that the all skeletal muscles are equally perfused during cycling.

The work of numerous investigators has shown that MBF increases in a linear manner with increasing work intensity (Radegran, 1997; Sullivan et. al., 1987; Saltin, 1985; Pirnay et. al., 1972; Grimby et. al., 1967). Sullivan et. al. (1987) found a near-linear relationship (r = 0.91) between leg blood flow, measured by thermodilution, and whole body oxygen consumption. In contrast, the results of the present study display poor to non-existent correlations between MBF and workload (see Table 3). The discrepancy between the clearance values and those obtained with other methods may be due to a diffusion limitation of [133]Xenon. It has been suggested that [133]Xenon clearance may be partially limited by diffusion (Bonde-Petersen et. al., 1975). Such a limitation would become greater at higher blood flows because of the shorter contact time between blood and tissue (Cerretelli et. al., 1984). This is an attractive explanation for the low MBF measured in the present study under maximal exercise conditions but it must be noted that not all researchers agree on this point (Clausen et. al., 1971; Pirnay et. al., 1972, Cerretelli, 1984). It has been shown that the maximal MBF achieved during exercise (measured by [133]Xenon clearance) can be exceeded if circulation is occluded in the opposite leg (Pirnay et. al., 1972) or if the MBF is measured after ischaemia (Clausen

et. al., 1971). This has been interpreted as meaning that a diffusional limitation does not exist because it is possible to induce higher MBF values than those achieved during maximal exercise. This argument appears to be somewhat flawed because what these findings may really mean is that the MBF achieved under maximal exercise conditions does not represent the highest possible MBF value of the ^{133}Xenon technique. That is to say that a diffusional limitation may result in an underestimation of MBF which would be larger in magnitude the greater the blood flow.

On the other hand, Cerretelli (1984) found no evidence for a diffusion limitation as the ratio between ^{133}Xenon MBF values and microsphere or directly recorded MBF values did not change across exercise conditions. However, he states that ^{133}Xenon may also be affected by venoarterial backdiffusion which would slow clearance rates particularly at low blood flows. Thus the combination of both effects could possibly act to reduce the clearance rate to a similar degree at all blood flow levels.

Even if such a limitation does exist, it would not explain the discrepancy between our results and those from other ^{133}Xenon studies which used the traditional method to monitor isotope clearance.

Another possible explanation for the low MBF values under maximal conditions involves the low initial counting rates which were obtained with the gamma camera. Because the majority of the isotope was washed out of the muscle within two to three minutes after the onset of exercise, it was necesary to use the first 90 seconds of clearance data to calculate MBF. If MBF has not reached its maximal value until after the majority of the isotope has been washed out, the MBF calculations may be measuring flow during

its rise to maximal values. This possibility is supported by a recent study which examined the temporal relationship between blood flow, blood pressure and muscle contractions (Radegran et. al., 1998). The increase in blood flow was characterized by a one component (~15% of peak power output, onset latency of ~0.3-5 seconds), two-component (~40-70% of peak power output, onset latency of ~4.2 seconds), or three-component exponential model (>75% of peak power output, onset latency of ~30 seconds). The initial elevetion in blood flow is facilitated by the muscle pump, whereas the second phase is controlled by potent vasodilatory factors. Blood flow was found to level off after ~10 seconds, 50-60 seconds, and 90-150 seconds at ~15%, ~40-70%, and >75% of peak power output, respectively. Thus it appears likely that MBF values in the present study at VO2max and possibly at the AT workload, were determined before a leveling off had occured.

If this is true, the higher MBF reported in the trained group at VO2max (p < 0.05) and at AT (p > 0.05) may be due to a faster response at the onset of exercise in trained individuals as reported by Shoemaker et. al. (1996).

5.2 Summary and Conclusions

The MBF results obtained using a gamma camera to measure isotope clearance, as described by Chung et. al. (1987), are not in agreement with the findings of many researchers. Given that BF supplies the muscle with oxygen and removes waste products, it would be expected to increase along with exercise intensity. This relationship has been shown by virtually all researchers who have examined MBF at various workloads

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(Radegran, 1997; Sullivan et. al., 1987; Saltin, 1985; Pirnay et. al., 1972; Grimby et. al., 1967). Such a relationship was not evident in the present study. The MBF measured at the two submaximal workloads is similar to that reported by other authors using [133]Xenon clearance, and if one multiplies these values by a correction factor of two, as suggested by Cerretelli (1984), the values are similar to those from thermodilution and ultrasound Doppler. The MBF values recorded under maximal conditions however, are not as high as those detected from other methods or from other [133]Xenon studies. This is likely due to a limitation of the gamma camera technique. When using a gamma camera to measure clearance, useful values are obtained only during the initial two to three minutes. Thus this technique may not be suitable for measuring high MBF's because such values are not attained during the first two minutes of exercise. The other limitation of the technique is its high variability. In order to detect small changes in MBF, a large number of subjects must be tested.

Despite these limitations, the [133]Xenon method still has useful applications. It is convenient to administer, non-invasive, and represents a valid source of information for estimating relative changes in blood flow.

REFERENCES

Armstrong, R. B. (1988). Magnitude and distribution of muscle blood flow in conscious animals during locomotory exercise. *Medicine and Science in Sports and Exercise*, 5, 119-123.

Bergman, H., Bjorntorp, P., Conradson, T. B., Fahlen, M., Stenberg, J., and Varnauskas, E. (1973). Enzymatic and circulatory adjustments to physical training in middle-aged men. *European Journal of Applied Physiology*, 3, 414-418.

Bonde-Petersen, F., Henriksson, J., and Lundin, B. (1975). Blood flow in thigh muscle during bicycle exercise at varying work rates. *European Journal of Applied Physiology*, 34, 191-197.

Cerretelli, P., Pendergast, D., Marconi, C., and Piper, J. (1986). Blood flow in exercising muscles. *International Journal of Sports Medicine*, 7, 29-33.

Cerretelli, P., Marconi, C., Pendergast, D., Meyer, M., Heisler, N., and Piper, J. (1984). Blood flow in exercising muscles by xenon clearance and by microsphere trapping. *Journal of Applied Physiology: Respiratory, Environmental, Exercise physiology*, 56(1), 24-30.

Chung, S. Y., Kim, I., Ryo, U. Y., Maskin, C., and Pinsky, S. (1987). Blood flow measurement in muscle with [133]Xenon. *Radiology*, 165, 571-572.

Clausen, J. P. and Lassen, N. A. (1971). Muscle blood flow during exercise in normal man studied by the [133]Xenon clearance method. *Cardiovascular Research*, 5, 245-254.

Clausen, J. P. (1976). Circulatory adjustments to dynamic exercise and effect of physical training in normal subjects and in patients with coronary artery disease. *Progressive Cardiovascular Diseases*, 18, 459-495.

Conn, H. L. (1961). Equilibrium distribution of radioxenon in tissue: xenon-hemoglobin association curve. *Journal of Applied Physiology*, 16, 1065-1070.

Corbally, M. T. and Brennan, M. F. (1990). Non-invasive measurement of regional blood flow in man. *The American Journal of Surgery*, 160, 313-321.

Elia, M. and Kurpad, A. (1993). What is the blood flow to resting human muscle? *Clinical Science*, 84, 559-563.

Frangolias, D. D. and Rhodes, E. C. (1995). Maximal and ventilatory threshold responses to treadmill and water immersion running. *Medicine and Science in Sports and Exercise*, 27, 1007-1013.

Gollnick, P. D. and Saltin, B. (1982). Significance of skeletal muscle oxidative enzyme enhancement with endurance training. *Clinical Physiology*, 2, 1-12.

Grimby, G., Haggendal, E., and Saltin, B. (1967). Local Xenon 133 clearance from the quadriceps muscle during exercise in man. *Journal of Applied Physiology*, 22(2), 305-310.

Hickner, R. C., Bone, D., Ungerstedt, U., Jorfeldt, L., and Henriksson, J. (1994). Muscle blood flow during intermittant exercise: comparison of the microdialysis ethanol technique and [133]Xenon clearance. *Clinical Science*, 86, 15-25.

Holloszy, J. O. (1973). Biochemical adaptations to exercise: Aerobic metabolism. *Exercise and Sport Science Reviews*, 1, 45-71.

Jorfeldt, L., and Wahren, J. (1971). Leg blood flow during exercise in man. *Clinical Science*, 41, 459-473.

Kety, S. S. (1949). Measurement of regional circulation by the local clearance of radioactive sodium. *American Heart Journal*, 38, 322-328.

Klausen, K., Secher, N. H., Clausen, J. P., Hartling, O., and Trap-Jensen, J. (1982). Central and regional circulatory adaptations to one-legged training. *Journal of Applied Physiology*, 52, 976-983.

Lassen, N. A., Lindbjerg, J., and Munck, O. (1964). Measurement of blood flow through skeletal muscle by intramuscular injection of Xenon-133. *Lancet*, 686-689.

Laughlin, M. H., and Korthuis, R. J. (1987). Control of muscle blood flow during sustained physiological exercise. *Canadian Journal of Sport Sciences*, 12(Suppl. 1), 77S-83S.

Leinonen, H., Salminen, S., and Peltokallio, P. (1978). Capillary permeability and maximal blood flow in athletes and non-athletes measured by local clearances of [133]Xenon and [131]I. *Scandinavian Journal of Clinical and Laboratory Investigations*, 38, 223-227.

Pirnay, F., Marechal, R., Radermecker, R., and Petit, J. M. (1972). Muscle blood flow during submaximum and maximum exercise on a bicycle ergometer. *Journal of Applied Physiology*, 32(2), 210-212.

Radegran, G. (1997). Ultrasound Doppler estimates of femoral artery blood flow during dynamic knee extensor exercise in humans. *Journal of Applied Physiology*, 83(4), 1383-1388.

Radegran, G., and Saltin, B. (1998). Muscle blood flow at the onset of dynamic exercise in humans. *American Journal of Physiology*, 274, H314-H322.

Rowell, L. B. (1974). Human cardiovascular adjustments to exercise and thermal stress. *Rhysiological Reviews*, 54, 75-159.

Rowell, L. B. (1986). Human Circulation Regulation during Physical Stress. Oxford University Press, New York.

Rowell, L. B. (1988). Muscle blood flow in humans: how high can it go? *Medicine and Science in Sports and Exercise*, 20(5), 97-103.

Saltin, B. (1985). Hemodynamic adaptations to exercise. *American Journal of Cardiology*, 55, 42-47.

Saltin, B. (1988). Capacity of blood flow delivery to exercising skeletal muscle in humans. *American Journal of Cardiology*, 62, 30E-35E.

Secher, N. H., Clausen, J. P., and Klausen, K. (1977). Central and regional circulatory effects of adding arm exercise to leg exercise. *Acta Physiologica Scandinavica*, 100, 288-297.

Shepherd, J. T. (1983). Circulation to skeletal muscle. In, *Handbook of Physiology*, American Physiological Society: Bethesda, Maryland.

Sheriff, D. D., Rowell, L. B., and Secher, A. M. (1993). Is rapid rise in vascular conductance at onset of dynamic exercise due to muscle pump? *American Journal of Physiology*, 265, H1227-H1234.

Shoemaker, J. K., Phillips, S. M., Green, H. J., and Hughson, R. L. (1996) Faster femoral artery blood velocity kinetics at the onset of exercise following short-term training. *Cardiovascular Research*, 31, 278-286.

Skinner, N. S. (1975). Skeletal muscle blood flow: metabolic determinants. In, *The Peripheral Circulations*, edited by Zelis, R., Grune and Stratten: New York, 57-78.

Snell, P. G., Martin, W. H., Buckey, J. C., and Blomqvist, C. G. (1987). Maximal vascular leg conductance in trained and untrained men. *Journal of Applied Physiology*, 62(2), 606-610.

Styf, J. (1990). The influence of external compression on muscle blood flow during exercise. *American Journal of Sports Medicine*, 18, 92-95.

Sullivan, M. J., Binkley, P. K., Unverferth, D. V., and Leier, C. V. (1987). Hemodynamic and metabolic responses of the exercising lower limb of humans. *Journal of Laboratory and Clinical Medicine*, 110, 145-152.

Terjung, R. L., McAllister R. M., and Mackie-Engbretson, B. (1990). The influence of exercise training on muscle blood flow. In, *International Perspectives in Exercise Physiology*, edited by Nazar, K., Human Kinetics Books: Champaign, Ill., 19-25.

Tonnesen, K. H. (1964). Blood flow through muscle during rhythmic contraction measured by [133]Xenon. *Scandinavian Journal of Clinical and Laboratory Investigation*, 16, 646-654.

Tonnesen, K. H. and Sejrsen P. (1970). Washout of [133]Xenon after intramuscular injection and direct measurement of blood flow in skeletal muscle. *Scandinavian Journal of Clinical and Laboratory Investigations*, 25, 71-81.

Varnauskas, E., Bjorntorp, P., Fahlen, M., Prerovsky, I., and Stenberg, J. (1970). Effects of physical training on exercise blood flow and enzymatic activity in skeletal muscle. *Cardiovascular Research*, 4, 418-422.

Vicini, P., Bonadonna, R. C., Utriainen, T., Nuutila, P., Raitakari, M., Yki-Jarvinen, H., and Cobelli, C. (1997). Estimation of blood flow heterogeneity distribution in human skeletal muscle from positron emission tomography data. *Annals of Biomedical Engineering*, 25, 906-910.

Volkov, N. I., Shirkovets, E. A., and Borilkevich, V. E. (1975). Assesment of aerobic and anaerobic capacity of athletes in treadmill running. *European Journal of Applied Physiology*, 34(2), 121-130.