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THE ROLE OF O_2 DELIVERY IN LIMITING THE IMMEDIATE ADJUSTMENT OF $\dot{V}O_2$ DURING THE TRANSITION FROM REST TO SUBMAXIMAL EXERCISE

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THE ROLE OF OXYGEN DELIVERY IN LIMITING THE IMMEDIATE ADJUSTMENT OF OXYGEN UPTAKE DURING THE TRANSITION FROM REST TO SUBMAXIMAL EXERCISE.

by
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ABSTRACT

THE ROLE OF OXYGEN DELIVERY IN LIMITING THE IMMEDIATE ADJUSTMENT OF OXYGEN UPTAKE DURING THE TRANSITION FROM REST TO SUBMAXIMAL EXERCISE.

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The purpose of this investigation was to determine the effects of increased and decreased O_2 delivery on the rate of adjustment of $\dot{V}O_2$ at the onset of aerobic exercise. Graded levels of lower body positive and negative pressure were used in an attempt to increase and decrease blood flow to working arm muscles. Seven healthy subjects performed a rest-to-exercise transition on an arm ergometer under each of five experimental pressures (-40, -20, +20, +40 mm Hg and ambient pressure). Each ten-minute bout of submaximal exercise was preceded by ten minutes of rest, divided into five minutes at ambient pressure and five minutes at the experimental pressure. Limb circumferences, heart rate, $\dot{V}O_2$, mean arterial pressure and forearm blood flow were measured. Application of lower body negative pressure resulted in graded translocation of blood volume from the upper to the lower body and graded increases in heart rate which reached statistical significance at -40 mm Hg. Mean arterial pressure and blood flow were not statistically altered, although blood flow decreased an average of -21.5% and -23.4%

at -20 and -40 mm Hg, respectively. Application of lower body positive pressure (+20 and +40 mm Hg) resulted in graded translocation of blood volume from the lower to the upper body. Heart rate and oxygen consumption were unaffected, while mean arterial pressure was greater than that seen at both -20 and -40 mm Hg. Decreases of -3.5% and -7.3% in forearm blood flow were detected at +20 and +40 mm Hg respectively. No statistically significant differences were detected in either τ or MRT, the kinetic parameters used to assess the rate of $\dot{V}O_2$ adjustment. Multiple correlation shows that experimental lower body pressure and forearm blood flow are more strongly correlated with MRT ($R=.45$, $P<0.05$) than they are with τ ($R=.28$, $P=0.26$). The coefficient of determination (R^2) indicates that the parameters experimental pressure and forearm blood flow together account for approximately 20% of the total variance in MRT. Individually, the contribution by forearm blood flow was statistically significant ($P<0.05$), but the contribution by experimental pressure was not.

DEDICATION

This dissertation is dedicated to the loving memory of my sister, Karen. Cancer intervened in her plans to accompany me to the end of this long journey. Her spirit, alive in my heart, carries out her wishes.

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CHAPTER I

THE PROBLEM

"Limited data are available to suggest that the kinetics of $\dot{V}O_2$ can be made faster if more O_2 is delivered.

R. Hughson, 1990

At submaximal exercise intensities, a steady-state oxygen uptake is achieved in which the energy needs of the working muscles primarily are met via the aerobic energy production system. However, at the onset of exercise and/or during the transition to an exercise load of higher intensity, there is a time lag before the rate of oxygen uptake ($\dot{V}O_2$) reaches this steady-state level. Since the energy needs are not met immediately by aerobic mechanisms, part of the energy needed during this period is supplied via nonaerobic mechanisms (e.g., the stored phosphagens and glycolytic breakdown to pyruvate).

The term "oxygen deficit" refers to the difference between the amount of oxygen (O_2) actually consumed during exercise and the amount that would have been consumed if steady state were reached immediately. As the workload becomes larger, the magnitude of this O_2 deficit increases (Hagberg, Hickson, Ehsani, & Holloszy, 1980), as does the contribution of energy from the nonaerobic mechanisms.

The term "O_2 uptake kinetics" refers to the rate of increase in O_2 consumption following exercise onset or workload transition as steady state is approached. During this period, O_2 uptake increases in an exponential manner (Cerretelli, Pendergast, Paganelli, & Rennie, 1979; Cerretelli, Sikand, & Farhi, 1966; di Prampero, Davies, Cerretelli, & Margaria, 1970; Henry, 1951; Pattengale & Holloszy, 1967; Whipp & Wasserman, 1972). Both the work intensity of the exercise and the training state of the individual performing the exercise affect the rate of adjustment to steady-state $\dot{V}_{O_2}$.

It seems reasonable that the time to reach steady state would be longer at heavier workloads than at lighter workloads due to the larger adjustments that are required in the circulatory, respiratory, and metabolic systems. Many studies have supported this hypothesis (Bason, Billings, Fox, & Gerke, 1973; Hagberg, et al., 1980; Hagberg, Nagle, & Carlson, 1978; Henry & DeMoor, 1956; Hickson, Bomze, & Holloszy, 1978; Linnarsson, 1974; Pendergast, Shindell, Ceretelli, & Rennie, 1980; Whipp & Wasserman, 1972); although some studies have shown an onset half-time of 30 seconds, regardless of the exercise intensity (Cerretelli, et al., 1966; di Prampero, et al., 1970; Henry, 1951; Margaria, Mangili, Cuttica, & Cerretelli, 1965).

Even though steady state O_2 consumption during submaximal work is unaffected by training status (Hagberg, et al., 1980; Hickson, et al., 1978; Karlsson, Nordesjo, Jorfeldt, & Saltin, 1972; Weltman & Katch, 1976), trained individuals adapt more quickly at the onset of exercise (i.e., reach steady state more rapidly) than do untrained individuals (Cerretelli, et al., 1979; Ebfeld, Hoffman, & Stegemann,

1987; Hagberg, et al., 1980; Hagberg, et al., 1978; Hickson, et al., 1978; Weltman & Katch, 1976). In addition, Karlsson et al. (1972) reported that the exercise-induced decrease in the ATP-PCr content of muscles is lessened and the exercise-induced increase in muscle lactate is attenuated following training. Both of these findings suggest a decreased reliance on anaerobic metabolism which would help to explain the phenomenon of a smaller O_2 deficit (i.e., more rapid O_2 uptake kinetics) in the trained state. However, this faster adjustment with training may be either a result of cellular adaptations that increase aerobic metabolism in the muscle or a result of increased O_2 transport capacity of the cardiovascular system and, therefore, the development of less muscle hypoxia.

Need for the Study

The physiologic mechanisms which may limit the rate of increase in O_2 uptake can be grouped into two categories: (a) O_2 delivery via the cardiovascular system and (b) O_2 utilization by the working muscles.

Oxygen Delivery

When exercise begins, adjustments in the circulatory system are necessary in order to increase blood flow, hence O_2 delivery, to the working muscles. These circulatory adjustments include not only an increase in cardiac output (the product of heart rate and stroke volume) but also a redistribution of blood flow from nonworking tissues (e.g., the gut) to working tissue (e.g., active muscle) (Rowell, 1974b). Because these cardiovascular adjustments do not occur

instantaneously, one can speculate that there may be a limit upon the immediate increase in O_2 uptake which can take place at exercise onset due to a restriction of O_2 at the cellular and ultimately the mitochondrial level.

Several studies support the theory that O_2 uptake kinetics are limited by O_2 delivery. For example, Hughson and Morrissey (1983) and Saltin (1969) have reported that the time courses for $\dot{V}O_2$ and heart rate adjustments at the onset of exercise, expressed both as a time constant (τ) and as the mean response time (MRT), are similar.

In other experimental studies that tend to confirm the dependent relationship between O_2 delivery and O_2 uptake kinetics, O_2 delivery to working muscle was altered by impairing cardiovascular function. ß-adrenergic blockade reduces heart rate, cardiac output, and mean arterial pressure (Epstein, Robinson, Kahler, & Braunwald, 1965) and blocks the receptors involved in vasodilatation of peripheral blood vessels (Brick, Glover, Hutchison, & Roddie, 1966). Collectively, O_2 delivery to working muscles is reduced as a result of impaired cardiovascular function. Administration of ß-blocking agents has resulted in slowed O_2 uptake kinetics (Hughson, 1984; Petersen, Whipp, Davis, Huntsman, Brown, & Wasserman, 1983; Twentyman, Disley, Gribben, Alberti, & Tattersfield, 1981).

Investigations in which the levels of inspired O_2 were altered also support the contention that O_2 delivery, not O_2 utilization, limits the rate of adjustment of O_2 uptake. Under hypoxic conditions, O_2 uptake kinetics are slowed (Linnarsson, Karlsson, Fagraeus, & Saltin, 1974; Murphy, Cuervo, & Hughson, 1989); but under hyperoxic

conditions, a significantly smaller O_2 deficit (i.e., faster O_2 uptake kinetics) is seen (Linnarsson, et al., 1974).

At the onset of exercise, blood flow is redistributed to working muscles from nonactive tissues. Hughson and Imman (1986) altered blood flow prior to supine arm exercise by occluding the circulation to nonworking leg muscle. Presumably, this procedure further reduced blood flow to nonactive tissues (i.e., the legs) while increasing blood flow to the arms. As hypothesized, the increase in O_2 delivery to the working arms resulted in faster O_2 uptake kinetics.

Oxygen Utilization

Other investigators have proposed that limitations of O_2 conductance/utilization distal to the capillary are the cause for the lag in the adjustment of $\dot{V}_{O_2}$ from rest to exercise (Cerretelli, et al., 1979; Pendergast, et al., 1980; Sahlin, Ren, & Broberg, 1988). If a slow adjustment of the respiratory and circulatory systems were limiting, a rapid transition from rest to exercise would result in a larger O_2 deficit than that experienced during a gradual transition. However, Sahlin et. al. (1988) saw no differences between gradual and rapid work transitions for either O_2 deficit or anaerobic energy utilization as estimated by PCr breakdown and lactate accumulation. The authors concluded that this evidence suggests O_2 delivery is not the mechanism which limits the rate of increase in O_2 uptake.

Possible mechanisms whereby O_2 utilization is limiting include: (a) capillary to muscle fiber O_2 exchange, (b) myoglobin concentrations, (c) O_2 diffusion distance to the mitochondria,

(d) mitochondrial density and therefore oxidative capacity, and/or
(e) a lag in the signal linking ATP demand with ATP supply.

When the adjustments of $\dot{V}O_2$, cardiac output ($\dot{Q}$), heart rate, and blood flow were determined under various conditions (arm and leg exercises at low and high workloads), cardiac output, heart rate, and blood flow always adjusted faster (i.e., had a smaller τ) than did $\dot{V}O_2$ (Pendergast, et al., 1980). Furthermore, under conditions where the time required for $\dot{V}O_2$ to reach steady state was prolonged (e.g., at high workloads and with arm exercise), the time required for $\dot{Q}$ to reach steady state was not as prolonged as that for $\dot{V}O_2$. These results comparing the central (cardiac output and heart rate) and peripheral (mean blood flow) circulatory adjustments with those observed in $\dot{V}O_2$ at the onset of exercise under various conditions suggest a limitation on O_2 uptake kinetics that is distal to the capillary bed (i.e., O_2 utilization).

Summary

Whether to attribute the limitation on the rate of adjustment of $\dot{V}O_2$ to O_2 delivery or O_2 utilization still is unknown. Experimentally, O_2 utilization has not been altered acutely in humans and, in fact, is probably impossible to modify without training. Altering O_2 delivery is another approach to solving this problem. Experimental evidence in which O_2 uptake kinetics are slowed when O_2 delivery to working muscles is impaired and O_2 uptake kinetics are accelerated when O_2 delivery is enhanced support the contention that O_2 delivery is the limiting factor. Decreasing O_2 transport by impairing cardiovascular function, restricting O_2 delivery via hypoxia, and altering blood flow

distribution does appear to slow O_2 uptake kinetics. Yet, as Hughson (1990) states, "Limited data are available to suggest that the kinetics of $\dot{V}O_2$ can be made faster if more O_2 is delivered."

Purpose of the Study

The purpose of this investigation was to determine the effects of increased and decreased O_2 delivery on the rate of adjustment of O_2 uptake at the onset of aerobic exercise. Lower body positive pressure (LBPP) was used as a means to increase blood flow and, therefore, O_2 delivery to working arm muscles. Lower body negative pressure (LBNP) was used to decrease blood flow and, therefore, O_2 delivery to working arm muscles. Specifically, this study was designed to answer the following questions:

1. Does increased O_2 delivery to working muscles speed the adjustment of O_2 uptake (as measured by tau and mean response time) at the onset of aerobic exercise?
2. Does decreased O_2 delivery to working muscles slow the adjustment of O_2 uptake (as measured by tau and mean response time) at the onset of aerobic exercise?

Research Hypotheses

1. As compared to values obtained at atmospheric pressure, the use of LBPP to enhance blood flow and therefore O_2 delivery to working arm muscles will accelerate the change in $\dot{V}O_2$ occurring at the onset of aerobic exercise.

2. As compared to values obtained at atmospheric pressure, the use of LBNP to reduce blood flow and therefore O_2 delivery to working arm muscles will slow the change in $\dot{V}_{O_2}$ occurring at the onset of aerobic exercise.

Research Plan

Seven healthy subjects were recruited to participate in this study. Each subject served as his/her own control and performed a rest-to-exercise transition on an arm ergometer under each of five experimental lower body pressures (-40, -20, +20, +40 mm Hg and ambient pressure). Each bout of exercise lasted ten minutes and was conducted at a constant submaximal workload of 25 watts. During each test session, the exercise bout was preceded by ten minutes of rest which was divided into the first five minutes at ambient pressure and the second five minutes at the experimental pressure. The test sessions were performed in random order on each of five different test days.

Heart rate and measures of gas exchange were monitored continuously throughout the test session. Blood flow and blood pressure were measured during rest, both at ambient pressure and again at the experimental pressure. Use of the arms for cycling precluded these determinations during exercise. Blood lactate was measured prior to exercise and two minutes following completion of the exercise bout.

Significance of the Problem

The results of this study provide insight into the unresolved question of whether O_2 delivery plays a role in limiting the immediate adjustment of $\dot{V}_{O_2}$ toward steady state following exercise onset.

The search for noninvasive measures of various human capacities and functions is ongoing. Currently, $\dot{V}_{O_{2max}}$ is considered the best noninvasive indicator of cardiovascular function; however, a maximal graded exercise test is required to obtain this measure. Maximal exercise often is contraindicated, especially for cardiac patients; therefore, the performance of this test may be a risk itself.

Endurance capacity, measured as time to exhaustion during a standardized bout of exercise, is considered a good noninvasive measure of the metabolic capacity of the muscles. However, this test is time consuming, highly dependent on motivational level, and also is dangerous for cardiac patients.

If the mechanism limiting O_2 uptake kinetics at the onset of exercise is identified, oxygen uptake kinetics could become an important, noninvasive measure of the underlying physiological function or capacity that is limiting. Whether this limit is cardiovascular function or metabolic capacity, the short, submaximal test required to obtain a measure of O_2 uptake kinetics may provide a safe and simple alternative to either a graded exercise test or an exercise test to exhaustion.

Limitations

Although this study was designed to determine if altering blood flow affects O_2 uptake kinetics, it does not exclude the possibility that O_2 utilization also may be involved in kinetic control. Lower body pressure alterations have unknown effects on metabolic and/or physiologic functions. For instance, alterations in pressure may affect the functioning of enzymes, the capillary exchange of O_2, O_2 diffusion distances and/or mitochondrial exchange ratios. Therefore, the results of this study can only support or refute the role O_2 delivery may have in kinetic control, but cannot exclude any role O_2 utilization may have. In addition, abdominal compression during pressure generation may cause alterations in blood flow by restricting venous return or may influence the perceived work intensity.

Under ideal conditions, blood flow would have been "set" at predetermined rates through the exercising muscles. This fine control would have required invasive methods which would have posed an undue danger to the exercising subjects. Therefore, the indirect method of altering forearm blood flow via alterations in lower body pressures was used.

Venous occlusion plethysmography was utilized as a technique to measure forearm blood flow. The nature of this technique made it impossible to actually measure forearm blood flow during arm exercise. Therefore, blood flow was measured just prior to the beginning of exercise. The possibility exists that the interaction of exercise and alteration in lower body pressure may have caused an unexpected change in forearm blood flow that was not detected. In

addition, this technique measures total limb blood flow which includes flow to nonworking muscles. Hence, effective working muscle blood flow was not measured directly and was assumed to be proportional to total limb blood flow.

Abbreviations

a-v O_2 Δ=aterial - venous oxygen difference

ADP=adenosine diphosphate

ATP=adenosine triphosphate

CO_2=carbon dioxide

Cr=creatine

DBP=diastolic blood pressure

HR=heart rate

LBNP=lower body negative pressure

LBPP=lower body positive pressure

MAP=mean arterial pressure

MRT=mean response time

O_2=oxygen

OBLA=onset of blood lactic acid

PCr=phosphocreatine

P_i=inorganic phosphate

$P_{I_{O_2}}$=partial pressure of inspired oxygen

$P_{a_{O_2}}$=partial pressure of arterial oxygen

$\dot{Q}$=cardiac output

SBP=systolic blood pressure

SV=stroke volume

τ=time constant

T_D=time delay

V_{CO_2}=volume of carbon dioxide produced

$\dot{V}_{CO_2}$=rate of carbon dioxide production

V_{O_2}=volume of oxygen utilized

$\dot{V}_{O_2}$=rate of oxygen uptake or oxygen consumption

$\dot{V}_{O_{2max}}$=maximum rate of oxygen uptake or oxygen consumption

V_E=volume of air expired

$\dot{V}_E$=rate of air expired

CHAPTER II

REVIEW OF RELATED LITERATURE

"Living organisms, like machines, conform to the law of the conservation of energy, and must pay for all their activities in the currency of metabolism"

E. Baldwin, 1967

The science of exercise physiology is, in large part, the study of thermodynamics. Originally, thermodynamics was developed as a study of steam engines. Today, exercise physiologists use the immutable principles of thermodynamics to study not inanimate objects, but the human machine. According to the first law of thermodynamics, energy is neither created nor destroyed but may be transformed from one form to another. Muscular exercise is a beautiful demonstration of this law in a living system: energy is obtained through ingested foods, stored in the form of chemical energy in all cells, changed into mechanical energy during muscular work, and lost ultimately as heat.

Bioenergetics of Muscular Exercise

Thermodynamics is a branch of the physical sciences that deals with energy changes. Its counterpart in the biological sciences that

deals with the transformation and use of energy by living cells is known as bioenergetics. Energy is used by living organisms to perform many types of biological work: (a) mechanical work for muscular contraction and cell division, (b) chemical work for biological syntheses, (c) electrical work for neurological signaling, and (d) osmotic work for molecular packaging against a concentration gradient.

During exercise, energy utilization is increased due to the increase in mechanical work performed by the muscles. Chemical energy for muscular contraction is supplied via three interrelated but different energy systems. The system that predominates at any given time depends upon the intensity and duration of exercise being performed. The phosphagenic system is the immediate source of energy for high-intensity exercise which, at maximum effort, can be carried on for no more than eight to ten seconds. Anaerobic glycolysis becomes the primary energy source for exercise which, when taken to exhaustion, lasts approximately thirty to sixty seconds. Aerobic metabolism is the ultimate source of energy for muscular contraction during low-intensity exercise which can be continued for long periods of time.

Phosphagenic System

Adenosine triphosphate (ATP) is a high-energy phosphate compound that resides in all muscle cells and supplies the chemical energy for muscular contraction. Hydrolysis of the terminal phosphate (Reaction A) releases energy which is used to "slide" the contractile

elements of muscle, actin and myosin, over each other resulting in sarcomere shortening and muscular contraction.

$$\text{(A) ATP} + H_2O \xleftarrow{\text{ATPase}} \text{ADP} + P_i + \textit{energy}$$

$$\Delta G^{\circ\prime} = -7.3 \text{ kcal/mole}$$

In living cells the concentrations of these metabolites, in addition to temperature, pressure, and pH, differ from standard conditions (1.0M, 25 °C, 1 atm, 7.0 pH). Therefore, the free energy released from ATP hydrolysis *in vivo* (ΔG) is approximately 1.5 to 2.0 times that released under standard conditions ($\Delta G^{\circ\prime}$).

The total pool of ATP stored in the muscle is quite small. With no other sources to resupply ATP, muscular contraction would stop very quickly because ADP cannot be used as an energy source by the contractile filaments. Fortunately, muscle cells contain another high-energy phosphate compound, phosphocreatine (PCr), which immediately regenerates ATP from ADP (Reaction B).

$$\text{(B) ADP} + \text{PCr} \xleftarrow{\text{creatine kinase}} \text{ATP} + \text{Cr}$$

$$\Delta G^{\circ\prime} = -10.3 \text{ kcal/mole}$$

Phosphocreatine stores, although approximately four times more plentiful than ATP stores, are also quite small. Together these phosphagens could supply energy for only a few seconds of strenuous muscular work even if the entire pool were available for hydrolysis. Therefore, to support longer durations of exercise, ATP is replenished via catabolism of energy-rich fuels stored in the body. Because ATP acts as an energy donor as well as an energy receiver (i.e., it links the

energy-using and energy-yielding functions of the cell), it is frequently referred to as the "energy currency" of biological systems (Lipmann, 1941). Depending on the intensity of the exercise, ATP is produced by substrate-level phosphorylation in the cytosol (i.e., anaerobic glycolysis), oxidative or substrate-level phosphorylation in the mitochondria (i.e., aerobic metabolism), or some combination of both.

Short-Term Anaerobic System

During exercise of high-intensity, at the onset of any exercise, whenever there is an increase in exercise intensity, and to some extent throughout all intensities of exercise, ATP is synthesized as a result of substrate phosphorylation during glycolysis/glycogenolysis. The carbohydrates, glucose and/or glycogen, are partially broken down in these multiple-step processes to release some of their stored energy. When the O_2 supply is inadequate or oxidative capacity is exceeded, formation of lactic acid occurs (reactions C and D).

$$\text{(C) glucose} + 2\text{ADP} + 2P_i \rightarrow 2\text{lactate} + 2\text{ATP} + H_2O$$

$$\text{(D) glycogen}_{(n+1)} + 3\text{ADP} + 3P_i \rightarrow \text{glycogen}_{(n)} + 2\text{lactate} + 3\text{ATP} + H_2O$$

Lactic acid formed via anaerobic glycolysis can be reoxidized in muscle or removed from the blood by the liver during and/or following exercise (Brooks, 1985). Lactate turn-over accelerates as exercise intensity increases; however, blood lactate levels do not increase immediately. Above intensities of about 55% of $\dot{V}O_{2max}$, lactate production begins to exceed lactate removal capabilities resulting in

accumulation in the blood. The point where lactate levels begin to rise is known as the lactate threshold or the onset of blood lactate accumulation (OBLA).

Long-Term Aerobic System

When O_2 supplies are adequate and the capacity of the oxidative enzymes are not exceeded, ATP is synthesized via complete oxidation of substrates (e.g., reactions involving the Krebs cycle, electron transport and oxidative phosphorylation). Unlike anaerobic glycolysis where only carbohydrate is used as a fuel source, aerobic pathways may "burn" carbohydrates, fats, and/or proteins. Figure 1 provides a simplified overview of the entry of these substrates into the metabolic machinery of the cell. Oxygen is the final electron acceptor of the entire aerobic system. The mitochondria must both receive and utilize O_2 in order for complete combustion to occur.

Total combustion of one glucose yields 38 ATP molecules (reaction E), considerably more than the two formed during anaerobic glycolysis. Fatty acids, the major energy source released from stored triglycerides (fat) are catabolized via ß-oxidation and yield approximately 3.5 times more ATP per molecule than glucose yields (reaction F). The contribution to ATP pools by amino acid oxidation is small; however alanine, an amino acid formed in muscle during exercise, travels in the blood to the liver for conversion to glucose. Thus the glucose-alanine cycle is an important gluconeogenic mechanism which helps to maintain blood glucose homeostasis during exercise. In fact, this cycle may contribute up to 5% of the total fuel during prolonged exercise (Felig & Wahren, 1971).

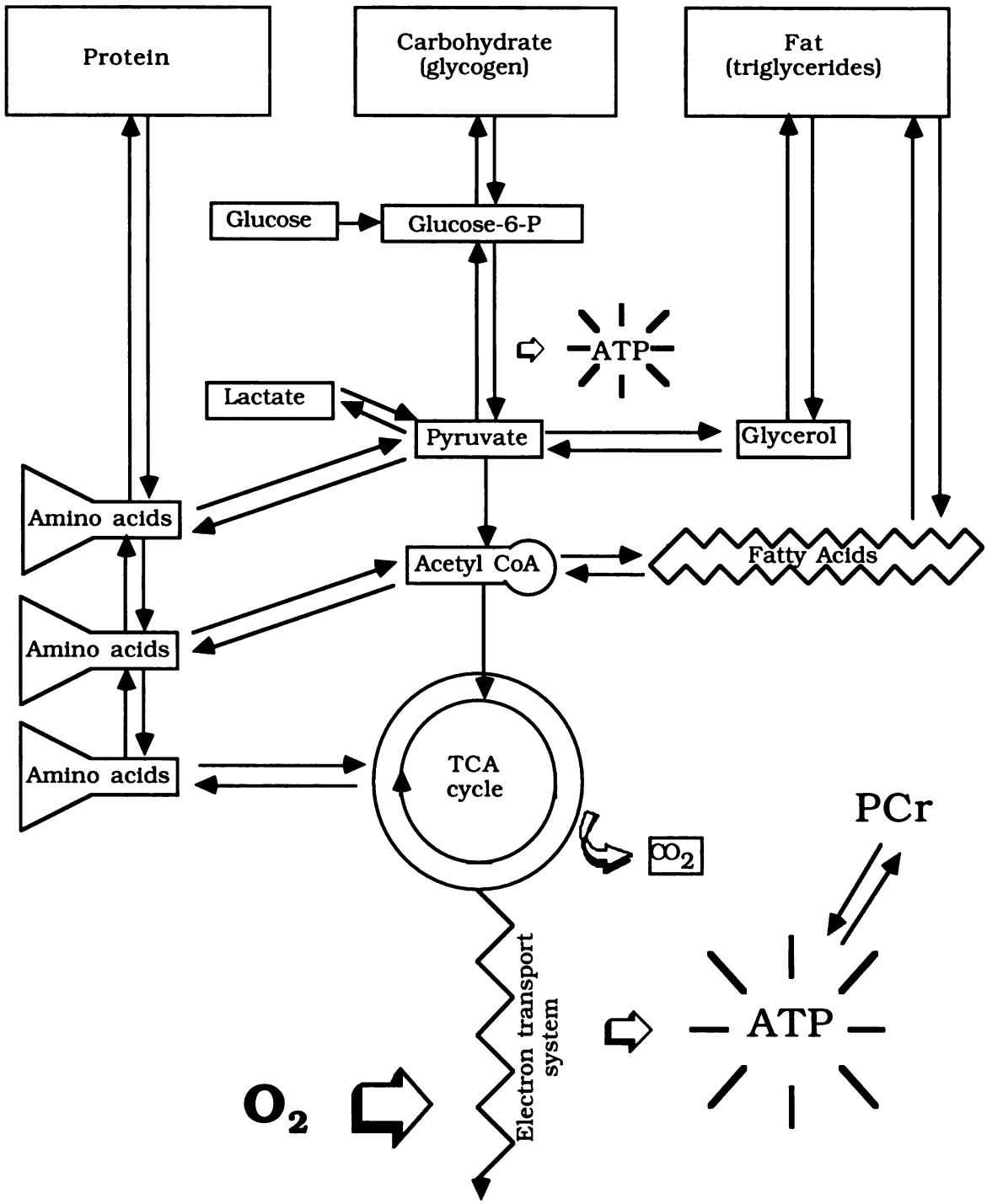


Figure 1. Overview of energy metabolism. Arrows do not necessarily represent a single step. Note convergence of all three fuel source pathways into one common pathway.

(E) glucose + 38ADP + 38P_i +6O_2 → 6CO_2 + 38ATP + 44H_2O

(F) palmitate + 129ADP + 129P_i +23O_2 → 16CO_2 + 129ATP + 16H_2O

($C_{16:0}$ fatty acid)

Measurement of Energy Expenditure During Exercise

Muscular work is accomplished as a result of the energy released via the the metabolic pathways just discussed. As this energy is converted from one form to another, the exchange is imperfect resulting in loss of mechanically useless heat energy called entropy (the second law of thermodynamics). All physiologic processes ultimately result in the loss of energy as heat. Measurement of the heat liberated due to biological combustions yields a direct measure of the energy expenditure of the organism.

Direct Calorimetry

Heat liberated by the burning of food in a bomb calorimeter is an index of the food's energy content. The amount of heat needed to raise one gram of water one degree Centigrade is known as a calorie. Similarly, heat liberated by a living organism due to "internal" combustion of foods is an index of the energy expenditure of an organism. To measure evolved heat directly, a human is placed inside a sealed chamber. Water surrounding the insulated chamber is warmed thereby reflecting the person's heat production.

At rest, the rate of heat production reflects the metabolic energy yield needed to sustain basic life processes. This rate can be measured quite accurately. During exercise, the rate of heat production increases, reflecting the increase in energy needed for muscular work.

However, measurement of heat production is impractical and also inaccurate during exercise. It is difficult, if not impossible, to distinguish between heat evolved by the body and that generated by the ergometer. In addition, not all heat produced is dissipated as is evidenced by a rise in core temperature during exercise. Therefore calorimeter responses alone are erroneous.

Indirect Calorimetry

During aerobic energy metabolism, the body utilizes O_2 and produces CO_2 for energy production. In fact, energy output is related quantitatively to the volumes of these respired gases. A typical mixed diet of carbohydrates, fats, and proteins liberates approximately 4.82 kcalories per liter of O_2 (Brooks & Fahey, 1985; McArdle, Katch, & Katch, 1986). Thus, by measuring the amount of O_2 consumed, the heat liberated can be calculated indirectly. The name indirect calorimetry falsely implies lesser quality when, in fact, O_2 consumption is a more useful, accurate, and easily obtainable measure than is heat evolution.

Indirect calorimetry does, however, have its limitations. As stated earlier, when ATP is supplied to contracting muscles via the phosphagenic and/or short-term energy systems, no O_2 is consumed. Therefore, during periods of short-term, high-intensity exercise, O_2 consumption values do not parallel energy expenditure. The most accurate estimates of energy expenditure occur at steady state workloads below those at which lactate begins to accumulate in the blood (approximately 55% of maximum O_2 uptake capacity).

Maximal Oxygen Consumption

Many times O_2 consumption data are not converted into caloric equivalents. Oxygen consumption expressed per unit of time (e.g. liters O_2/min or ml O_2/kg body weight/min) is itself a useful quantity and is actually a power measure. It responds in a positive, linear fashion to progressive, continuous increases in workload. The value at which O_2 consumption no longer increases even with an increase in workload is termed maximal O_2 uptake or maximal aerobic power. It is a quantitative representation of a person's maximal capacity for aerobic synthesis of ATP.

The Fick equation defines the relationship between O_2 consumption ($\dot{V}_{O_2}$), cardiac output ($\dot{Q}$) and the arterio-venous O_2 difference (a-v O_2 Δ) at maximum exercise:

$$\dot{V}_{O_2max} = (\dot{Q}_{max}) * (\text{a-v } O_2\ \Delta_{max}).$$

Hill and Lupton (1923) first suggested that $\dot{V}_{O_2max}$ is limited by the O_2 transport capacity of the cardiovascular system. This is in contrast to the concept that $\dot{V}_{O_2max}$ is limited by the ability of working muscles to oxidize substrate (Blomqvist & Saltin, 1983; Rowell, 1974b). The immediate limiting factor in the functional capacity of the cardiovascular system appears to be the ability to achieve a large stroke volume (SV) which, in turn, limits cardiac output (Blomqvist & Saltin, 1983; di Prampero, 1985; Saltin, 1990).

$$\dot{Q}_{max} = SV_{max} * HR_{max}$$

For this reason, maximal O_2 consumption is sometimes referred to as the best measure of cardiovascular fitness.

Submaximal Oxygen Consumption

At submaximal exercise intensities, a steady state O_2 consumption is achieved in which the energy needs of the working muscles are met via the energy production systems already discussed. Prolonged exercise at these submaximal workloads cannot proceed indefinitely however, and eventually ceases. Endurance capacity, defined as time to exhaustion, is limited in this instance not by O_2 delivery, but rather by substrate availability in the working muscles (Blomqvist & Saltin, 1983; Holloszy & Coyle, 1984).

Oxygen consumption is not increased at submaximal workloads following training (Hagberg, et al., 1980; Hickson, et al., 1978; Karlsson, et al., 1972); however, lactate levels are decreased (Karlsson, et al., 1972), and glycogen stores are depleted less rapidly (Hermansen, Hultman, & Saltin, 1967). These adaptations are most likely due to an increase in fatty acid oxidation as evidenced by a decrease in the respiratory exchange ratio (Holloszy, 1973) made possible by increases in mitochondrial volumes (Morgan, Cobb, Short, Ross, & Gunn, 1971) and ß-oxidation enzymes (Costill, Fink, Getchell, Ivy, & F.A., 1979; Jansson & Kaijser, 1977; Orlander, Kiessling, Karlsson, & Ekblom, 1977; Schantz, Henriksson, & Jansson, 1983). Cumulatively, these adaptations improve endurance capacity by attenuating the reliance on anaerobic and aerobic glycolysis and preferentially increasing the use of fats to delay the onset of

performance limitations due to glycogen depletion and/or lactate accumulation (Blomqvist & Saltin, 1983).

Oxygen Consumption at the Onset of Exercise

At the onset of exercise and/or during the transition to an exercise load of higher intensity, the increased energy needs are not met immediately by aerobic supply mechanisms. This is apparent by the typical time lag following exercise onset or transition before the rate of O_2 consumption reaches a steady-state level. The difference between the amount of O_2 actually consumed during exercise and the amount that would have been consumed if steady state were reached immediately is known as the "O_2 deficit" (see Figure 2). Whereas the term "O_2 deficit" is associated with a quantitative amount of O_2, the term "O_2 uptake kinetics" refers to the rate of increase in O_2 consumption as steady state is approached (e.g., during the period immediately following exercise onset or transition).

Oxygen Deficit

The rate of energy production required to exercise at a given workload is constant. Since aerobic mechanisms cannot adjust immediately as is seen by the lag in O_2 consumption between exercise onset and steady state, part of the energy needed during this transition is supplied via nonaerobic mechanisms (e.g., the stored phosphagens and glycolytic breakdown to pyruvate).

The greater the workload, the greater the O_2 deficit (Hagberg, et al., 1980). As O_2 deficit increases, so does the contribution of energy from the nonaerobic mechanisms. The ATP-PCr content of

muscle decreases and the lactate concentration in muscle increases as the O_2 deficit becomes larger (Pernow & Karlsson, 1971). This suggests simultaneous contributions by all three energy systems, not an either-or, on-off energy supply mechanism.

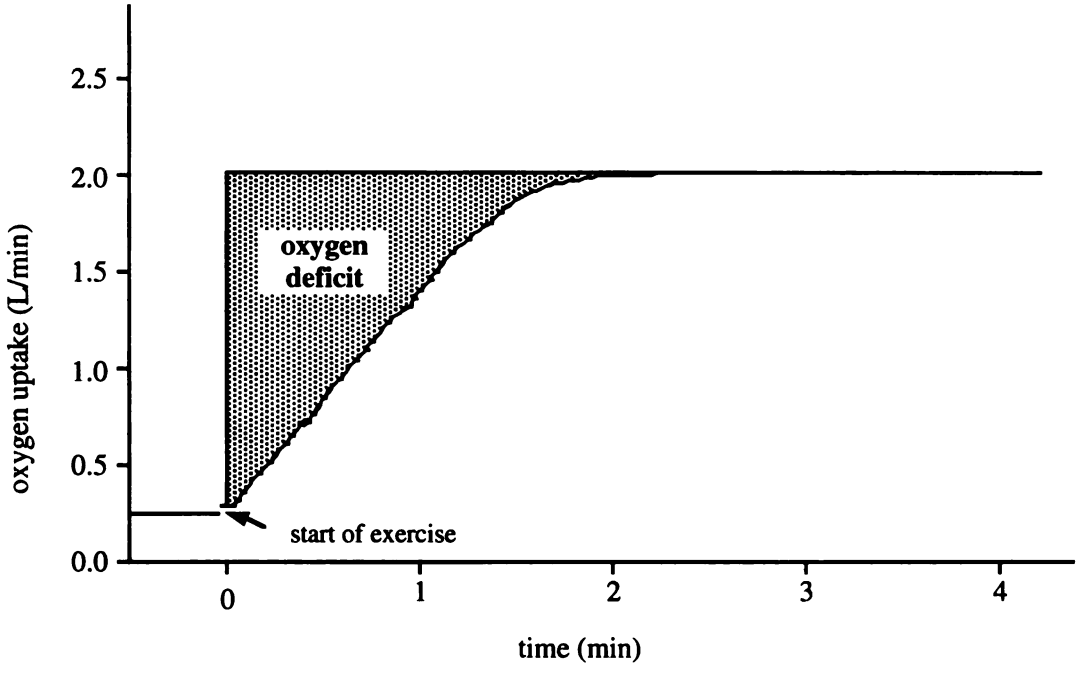


Figure 2. Oxygen uptake at the onset of exercise.

Oxygen Uptake Kinetics

Oxygen uptake increases in an exponential manner at the onset of exercise (Cerretelli, et al., 1979; Cerretelli, et al., 1966; di Prampero, et al., 1970; Henry, 1951; Pattengale & Holloszy, 1967; Whipp & Wasserman, 1972). This rate of adjustment to steady-state $\dot{V}O_2$ varies according to the work intensity used and to the training state of the individual performing the exercise.

Training Effects

Several investigators have shown that trained individuals adapt more quickly at the onset of exercise (e.g., reach steady state more rapidly) than do untrained individuals (Cerretelli, et al., 1979; Ebfeld, et al., 1987; Hagberg, et al., 1980; Hagberg, et al., 1978; Hickson, et al., 1978; Weltman & Katch, 1976), even when steady-state O_2 consumption is similar in both groups (Hagberg, et al., 1980; Hickson, et al., 1978; Karlsson, et al., 1972; Weltman & Katch, 1976). Karlsson et al. (1972) have shown that the exercise-induced decrease in the ATP-PCr content of muscles is lessened and the exercise-induced increase in muscle lactate is attenuated following training. This fact suggests that reliance on anaerobic metabolism is decreased which helps to explain the finding of a smaller O_2 deficit (e.g., more rapid O_2 uptake kinetics) in the trained state. However, this faster adjustment in $\dot{V}_{O_2}$ with training may be either a result of cellular adaptations that increase aerobic metabolism in the muscle as discussed in a previous section, or a result of the increased O_2 transport capacity of the cardiovascular system and, therefore, the development of less muscle hypoxia (Hagberg, et al., 1978). Similarly, increases in $\dot{V}_{CO_2}$, $\dot{V}_E$, and heart rate at the onset of exercise are significantly faster in trained than in untrained subjects (Hagberg, et al., 1980)

Workload Effects

Larger adjustments are required in the circulatory, respiratory, and metabolic systems at higher than at lower intensities of work. Therefore, it seems reasonable that, within any given subject, the time to reach steady state would be longer at higher workloads. This

contention is supported by many investigators (Bason, et al., 1973; Hagberg, et al., 1980; Hagberg, et al., 1978; Henry & DeMoor, 1956; Hickson, et al., 1978; Linnarsson, 1974; Pendergast, et al., 1980; Whipp & Wasserman, 1972); although some studies have demonstrated an onset half-time of 30 seconds, regardless of the exercise intensity (Cerretelli, et al., 1966; di Prampero, et al., 1970; Henry, 1951; Margaria, et al., 1965). Not surprisingly, the time course for increases in $\dot{V}_{CO_2}$, $\dot{V}_E$, heart rate, and cardiac output also are longer at heavier workloads (Broman & Wigertz, 1971; Hagberg, et al., 1980; Jones, Finchum, Russell, & Reeves, 1970).

Mechanisms Limiting Oxygen Uptake

Hughson (1990) suggests that the rate of increase in O_2 uptake under varying conditions provides information about the physiologic mechanisms that control this rate of increase. Possible limiting mechanisms can be grouped into two categories: (a) O_2 delivery and (b) O_2 utilization.

Oxygen Delivery

At the onset of exercise, circulatory adjustments commence in order to increase blood flow, hence O_2 delivery, to the working muscles. This increase in perfusion of active muscle tissue depends not only on an increase in cardiac output (the product of heart rate and stroke volume) but also on a redistribution of blood flow from nonworking areas via increased vascular resistance (vasoconstriction) to working muscle via decreased vascular resistance (vasodilatation). The vasoconstriction in nonworking areas allows a greater percentage

of the simultaneously increased cardiac output to perfuse the working muscle (Rowell, 1974b). This cardiovascular adjustment does not occur instantaneously, thereby temporarily restricting O_2 supply at the cellular level and ultimately at the mitochondria. Thus one can postulate a limit upon the instantaneous increase in O_2 uptake which can take place at exercise onset. Note that this initial O_2 limitation would be at the cellular level. The term suggested by Connett, Honig, Gayeski and Brooks (1990) for this state is adapted cell hypoxia as opposed to hypoxia (low $P_{I_{O_2}}$) or hypoxemia (low $P_{a_{O_2}}$).

Serendipitous evidence seems to support the theory that O_2 uptake kinetics are limited by O_2 delivery. That is, the time courses for $\dot{V}_{O_2}$ and heart rate adjustments to occur at exercise onset under varying conditions, expressed as both time constants (τ) and mean response times (MRT), are similar (Hughson & Morrissey, 1983; Saltin, 1969).

Experimental evidence also exists for O_2 delivery being a limiting factor. Several studies have shown slowed O_2 uptake kinetics following administration of ß-adrenergic blocking agents (Hughson, 1984; Petersen, et al., 1983; Twentyman, et al., 1981). ß-adrenergic blockade reduces heart rate, cardiac output, and mean arterial pressure (Epstein, et al., 1965) and blocks the receptors involved in vasodilatation of peripheral blood vessels (Brick, et al., 1966). Collectively, O_2 delivery to working muscles is reduced. Peterson's data (1983) suggest that there is not a direct ß-blockade depressant effect on the ventilatory control system during exercise, thus the

slowing of V_{O_2} kinetics probably is a result of the altered cardiovascular effects of ß-blockade.

Other studies have shown slowed O_2 uptake kinetics during exercise in hypoxic conditions (Linnarsson, et al., 1974; Murphy, et al., 1989). Linarsson et al. (1974) calculated a significantly larger O_2 deficit at an O_2 partial pressure (P_{IO_2}) of 99 mm Hg than at normoxia (149 mm Hg). Therefore, the estimated time course for $\dot{V}_{O_2}$ adjustment was longer when O_2 was restricted. This slowing of O_2 uptake kinetics during submaximal exercise was accompanied by increases in muscle and blood lactate concentrations and decreases in working muscle phosphocreatine. Accumulation of pyruvate in the working muscle was negligible; therefore, adapted cell hypoxia or an altered redox ratio seem to be more plausible explanations for increased lactate formation than does an overflow of glycolysis (Linnarsson, et al., 1974). Murphy et al. (1989) also have shown a slowing of O_2 uptake kinetics under hypoxic versus normoxic conditions during a submaximal step (abrupt) increase in workrate, but not during a ramp (gradual) increase in workrate. However, hypoxia did cause a decrease in $\dot{V}_{O_{2peak}}$ and absolute ventilatory threshold during both exercise protocols. The authors concluded that the ramp test is useful for determination of $\dot{V}_{O_{2max}}$ and ventilatory threshold, but due to lack of system linearity during this protocol, it is not suitable for determination of O_2 uptake kinetics.

Conversely, a significantly smaller O_2 deficit was seen during hyperoxic exercise (Linnarsson, et al., 1974). This faster adjustment

of $\dot{V}O_2$ was accompanied by smaller levels of muscle and blood lactate than those seen under hypoxic conditions.

The results of experiments in which kinetics are changed by altering the levels of inspired O_2 can be viewed collectively. Studies of both hypoxic and hyperoxic exercise support the contention that O_2 delivery, not O_2 utilization, limits the rate of adjustment of O_2 uptake.

As mentioned previously, at the onset of exercise blood flow is redistributed to working muscles from nonactive tissues. Hughson and Imman (1986) altered blood flow prior to supine exercise by occluding the circulation to nonworking leg muscle. This procedure reduced venous volume in the legs, thus presumably increasing effective central blood volume and permitting a relative increase in cardiac output and arm blood flow. As hypothesized, this increase in blood flow to the working arms resulted in faster O_2 uptake kinetics due to an assumed improvement in O_2 delivery.

Oxygen Utilization

Several investigators have proposed that O_2 transport is not limiting, but that factors related to local O_2 conductance/utilization distal to the capillary are responsible for the lag in the adjustment of $\dot{V}O_2$ from rest to exercise (Cerretelli, et al., 1979; Pendergast, et al., 1980; Sahlin, et al., 1988). The site of the limitation may be: (a) capillary to muscle fiber O_2 exchange, (b) myoglobin concentration, (c) O_2 diffusion distance to the mitochondria, (d) mitochondrial density and therefore oxidative capacity, and/or (e) a lag in the signal linking ATP demand with ATP supply.

If a slow adjustment of the respiratory and circulatory systems were limiting, a gradual transition from rest to exercise would diminish the O_2 deficit as compared to that experienced during a rapid transition. However, Sahlin et al. (1988) saw no differences between gradual and rapid work transitions in either O_2 deficit or anaerobic energy utilization as estimated by PCr breakdown and lactate accumulation. They concluded that the hypothesis of $\dot{V}O_2$ being limited by O_2 transport at exercise onset is not tenable.

In experiments where adjustments of $\dot{V}O_2$, cardiac output ($\dot{Q}$), and heart rate were determined during various conditions (arm and leg exercises at low and high workloads), cardiac output and heart rate always adjusted faster (i.e., had a smaller $\tau_{1/2}$) than did $\dot{V}O_2$ (Pendergast, et al., 1980). Furthermore, under conditions where the time required to reach steady-state $\dot{V}O_2$ was prolonged (e.g., at high workloads and with arm exercise), the difference in time between the $\tau_{1/2}\,\dot{V}O_2$ and the $\tau_{1/2}\,\dot{Q}$ became more pronounced. Therefore, the authors concluded that cardiac output does not limit the adjustment of $\dot{V}O_2$ in normal subjects.

These same authors used the ^{133}Xe clearance technique as an indicator of peripheral circulation under similar experimental conditions. Washout of the radioactive ^{133}Xe injected intramuscularly begins immediately, with an increased rate of washout occurring during exercise using the injected muscles. Again, during each of the various experimental conditions, the time to reach a faster steady-state mean blood flow was always less than the $\tau_{1/2}$ for $\dot{V}O_2$ to reach

steady state. Furthermore, under conditions where O_2 uptake kinetics become slower, mean blood flow was unaffected.

In summary, the results comparing the central (cardiac output and heart rate) and peripheral (mean blood flow) circulatory adjustments with those observed in $\dot{V}O_2$ at the onset of exercise under various conditions suggest a limitation on O_2 uptake kinetics that is distal to the capillary bed. Therefore, O_2 utilization, not O_2 delivery, is implicated in these studies.

Cardiovascular Responses to Blood Volume Redistribution

The technique of altering the pressure surrounding the lower portion of the body is commonly used to redistribute blood within the body. Cardiovascular responses during exposure of the lower body to subatmospheric pressures are similar to those seen with a shift from a supine to an upright position (Wolthuis, Bergman, & Nicogossian, 1974). Typically, the bottom portion of a supine subject is placed inside a solid wooden box. A tightly fitting rubber diaphragm, placed at the level of the iliac crest, creates a sealed chamber surrounding the lower portion of the body. Pressures within the chamber can be altered by application of either a suction (negative pressure) or a compression (positive pressure).

Lower Body Negative Pressure

Application of lower body negative pressure (LBNP) causes pooling of blood in the lower extremities and therefore, a decrease in central venous pressure (Johnson, Rowell, Niederberger, & Eisman, 1974; Victor & Leimbach, 1987). This pooling of blood in the legs

diminishes venous return to the heart which results in a decreased stroke volume, even at pressures as small as -20 mm Hg (Eiken & Bjurstedt, 1985; Nishiyasu, Xiangrong, Gillen, Mack, & Nadel, 1993). In an attempt to maintain cardiac output, heart rate increases (at negative pressures $\geq$20 mm Hg) (Eiken, 1988; Eiken & Bjurstedt, 1985; Eiken, Lind, & Bjurstedt, 1986; Lundvall & Edfeldt, 1994; Stevens & Lamb, 1965), but is inadequate in preventing a significant decrease in cardiac output (Eiken & Bjurstedt, 1985). Despite the decrease in $\dot{Q}$, compensating mechanisms are sufficient to prevent a loss of consciousness via maintenance of adequate cerebral blood flow (Stevens & Lamb, 1965).

At rest, forearm volume decreases and calf volume increases with the application of LBNP (Essandoh, Houston, Vanhoutte, & Shepherd, 1986). Forearm blood flow is progressively reduced with increasing negative pressures (by approximately 10%, 30%, 35%, 40% and 60% at -5, -10, -15, -20 and -40 mm Hg respectively). Although calf blood flow is unaltered at low levels of negative pressure, at larger negative pressures calf blood flow also is reduced (by approximately 15% and 60% at -20 and -40 mm Hg respectively) (Essandoh, et al., 1986). Generally, stable flow levels are established within 30 seconds following application of LBNP (Lundvall & Edfeldt, 1994). Lundvall and Edfeldt (1994) have shown that the progressive decreases in forearm blood flow parallel progressive increases in forearm vascular resistance with increasing negative pressure. In fact, an increase in peripheral resistance is an important component of a person's ability

to maintain adequate blood pressure in spite of diminished cardiac output (Stevens & Lamb, 1965).

Tolerance to negative pressure varies according to the duration and amount applied. In one study, by -80 mm Hg all subjects developed symptoms of impending syncope (Stevens & Lamb, 1965). Clinical symptoms of presyncope include pallor, dizziness, sweating and/or nausea associated with a precipitous drop in blood pressure, the severe narrowing of pulse pressure, and/or sudden bradycardia. These symptoms disappear rapidly when atmospheric pressure is reestablished in the chamber. Presyncopal symptoms were absent in all subjects at -25 mm Hg over a 20-minute period. At -40 and -60 mm Hg, 58% and 70% of subjects, respectively, became presyncopal with a mean duration before onset of symptoms of 8.3 minutes at -40 and -60 mm Hg.

Lower body negative pressure alters cardiovascular and ventilatory responses during supine leg exercise. Although resting heart rates were higher during LBNP than under normal atmospheric conditions, during mild to moderate exercise they were similar to those seen at atmospheric pressures (Eiken, 1988; Eiken & Bjurstedt, 1985; Eiken, et al., 1986). Nishiyasu et al. (1993) showed that during dynamic exercise there is an attenuation of the decrease in stroke volume which accompanies application of LBNP as compared to that seen with no exercise. He attributes this smaller decrement to an increased venous return caused by the muscle pumping action. Cardiac output is depressed by approximately two to three liters per minute both at rest and during exercise when LBNP (-40 mm Hg) is

applied (Eiken, 1988; Eiken & Bjurstedt, 1985). This decrement in cardiac output is similar to that seen when leg exercise is performed in an upright rather than a supine position over a wide range of workloads (Bevegard, Holmgren, & Jonsson, 1960; Bevegard, Holmgren, & Jonsson, 1963).

At rest, $\dot{V}_I$, $\dot{V}_{O_2}$ and blood lactate are unaffected by LBNP, although as exercise intensity increases, these variables consistently rise at a slower rate under subatmospheric pressures than at atmospheric conditions (Eiken, 1988). The authors attribute reduced exercise ventilation, lowered blood lactate levels and improved work performance to a more efficient blood perfusion of working leg muscles during LBNP.

Lower Body Positive Pressure

Few articles have examined the effects of supraatmospheric pressure on the cardiovascular and respiratory systems. Eiken and Bjurstedt (1987; 1988) reported no changes in resting heart rate or ventilation following application of 50 mm Hg of lower body positive pressure (LBPP). However, resting blood pressure (systolic, mean and diastolic), stroke volume, cardiac output and $\dot{V}_{O_2}$ were increased significantly. Increases in $\dot{V}_{O_2}$ were attributed to the added work performed by postural muscles in stabilizing the trunk against the upward pushing force of LBPP.

Dynamic exercise performance was impaired by 40% during LBPP with subjects discontinuing exercise due to working leg muscle fatigue (Eiken & Bjurstedt, 1987). This finding is supported by the fact that blood lactate concentrations rose more rapidly and were

significantly different from control values (obtained at atmospheric pressures) during all incremental work intensities. Although blood flows were not actually measured, high blood lactate levels, systolic arterial pressures and ventilations during exercise at supraatmospheric pressures support the contention of reduced leg muscle blood flows resulting from application of LBPP.

Other indirect evidence suggests that forearm blood flow may be increased with LBPP. Under atmospheric pressure, raising the legs of a recumbent subject increases intrathoracic blood volume and causes a reflex vasodilation in forearm muscle blood vessels resulting in increases in forearm blood flow (Roddie & Shepherd, 1963). This vasodilation appears to be mediated via activation of cardiopulmonary low-pressure receptors in response to increased central venous pressure. The LBPP-induced increase in stroke volume at rest suggests an increase in intrathoracic blood volume resulting from upward redistribution of blood from the lower extremities as well. This central fluid shift should lead to forearm vasodilation and increased blood flow as observed by Roddie and Sherperd (1963) in subjects with similar fluid shifts.

In a more recent study by Shi, Crandall and Raven (1993), application of lower body positive pressure to +20 mm Hg caused translocation of blood volume to the thoracic compartment, thereby increasing central venous pressure (CVP). This increase in CVP resulted in a significant rise in cardiac output which was accounted for by a significant rise in stroke volume but no change in heart rate. Mean arterial pressure and forearm blood flow were unaffected at this

pressure. At +40 mm Hg, CVP continued to rise, although cardiac output and stroke volume plateaued. No significant change in heart rate occurred. At this pressure, forearm blood flow and mean arterial pressure were significantly increased above those at ambient pressure. Conversely, Bevegard et al. (1977), using only +40 mm Hg, found no changes in stroke volume, cardiac output or heart rate. As in the previously cited study, forearm blood flow and mean arterial pressure were significantly increased.

Since application of supraatmospheric pressure causes an upward fluid shift, central blood volume is increased. Therefore, cardiovascular complications, such as those seen during LBNP, are not present. No clinical symptoms or complications were reported in the studies reported here, even at pressures as high as +50 mm Hg.

CHAPTER III

RESEARCH METHODS

"Much of our present knowledge about the physiology of exercise is derived from studies of the changes occurring during the unsteady states following onset of work or during recovery"

D. Linnarsson, 1974

During submaximal muscular exercise, respiratory and circulatory adjustments occur to maintain a remarkably constant internal environment despite large increases in metabolic rate. However, respiratory and/or cardiovascular measurements made only during steady state exercise usually are insufficient to explain the regulatory mechanisms which control these adjustments. More useful information regarding physiologic control mechanisms can be obtained during nonsteady state exercise; e.g., during the transitions between rest and exercise.

This study was designed to investigate one of the mechanisms which may limit the change in O_2 uptake as it approaches steady state following the transition from rest to exercise. Mechanisms which are thought to affect the rate of this change can be grouped into two categories: (a) O_2 delivery and (b) O_2 utilization. In this study, blood flow, hence O_2 delivery to working arm muscles, was altered using a

lower body pressure chamber. It was hypothesized that increasing blood flow to the arms (via lower body positive pressure) would result in a faster rate of O_2 adjustment and that decreasing blood flow to the arms (via lower body negative pressure) would result in a slower rate of O_2 adjustment. These results, if obtained, would support the theory that O_2 delivery is involved in limiting the immediate adjustment of $\dot{V}_{O_2}$ to steady state at the onset of submaximal exercise.

Subjects

Seven healthy subjects, without regard to sex, were recruited to participate in the study. Each subject completed a medical history form (see Appendix A) and was screened for possible cardiovascular and/or respiratory disease before final acceptance into the study. The experimental procedures and any possible risks were explained thoroughly to the subjects who were required to sign informed consent forms (see Appendix B) approved by the Human Subjects Committees of both Michigan State University and Alma College prior to any further participation in the study. To ensure familiarity with the equipment and experimental procedures, each subject was given an orientation to the lower body pressure chamber and learned to pedal an arm ergometer at a cadence set by a metronome. The subjects were instructed to refrain from eating for at least three hours before arriving in the laboratory and from exercising prior to being tested on any day that an experimental session was scheduled.

Experimental Apparatus

Experiments were conducted on supine subjects whose lower bodies were enclosed within an air-tight pressure chamber. The waist was sealed at the level of the naval with a tightly fitting rubber diaphragm. A PVC tube, which was permanently fixed inside the chamber, passed between the subject's legs at the level of the crotch to prevent downward displacement of the body during application of lower body negative pressure. Shoulder straps prevented upward displacement of the body during application of lower body positive pressure.

A pump, regulated by a rheostat, was used to maintain pressures inside the pressure chamber. One end of a hose was attached to the suction port of the pump and the other end was inserted into a hole in the chamber. This arrangement produced lower body negative pressure (LBNP) inside the chamber. When the hose was attached to the exhaust port of the pump, lower body positive pressure (LBPP) was produced inside the chamber. Stable pressure readings (both positive and negative) were obtained inside the chamber within five to ten seconds following initial start up of the pump.

Mild arm exercise was performed on a Monarch arm ergometer located on a wooden stand outside the pressure chamber. The ergometer was located in a comfortable cranking position, slightly above the subject's chest, and stabilized to prevent extraneous movements of the ergometer.

Experimental Design

Each subject was tested under all experimental conditions, thus serving as his/her own control. The study was conducted as a repeated measures design with rest-to-exercise transition occurring under each of five experimental lower body pressures (-40, -20, +20, and +40 mm Hg, as well as at ambient pressure). More severe negative pressures were not used in order to avoid possible problems with syncope. Uncomfortable abdominal compression and potential seal problems with the pressure chamber precluded use of higher positive pressures.

Each test session was conducted as illustrated in Figure 3 and lasted for a total of twenty minutes. For the first five minutes, the subject rested in the chamber at ambient pressure. At the beginning of minute six, the treatment pressure designated for the test session was applied. This pressure then was maintained by continuous pump action for the remainder of the session. During minutes six through ten, the subject performed no exercise but was under the influence of the experimental pressure. Exercise commenced at the beginning of minute eleven and lasted for the remaining ten minutes. Approximately five seconds before the beginning of exercise, technicians began to pedal the arm ergometer at a cadence of 50 revolutions per minute (a 25-watt workload). Therefore, at the start of exercise, the subject did not have to overcome the inertia of the flywheel. Higher workloads previously were determined to be too severe for some subjects to continue for the entire ten-minute bout.

Each subject completed only one exercise bout per day. The test sessions for each subject were scheduled with a minimum of 20 hours between visits. All treatments were completed in randomized order.

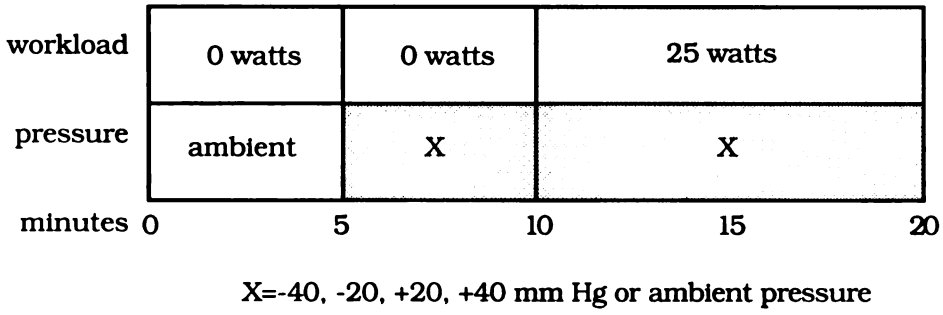


Figure 3. Individual exercise bout protocol.

Blood Flow Determinations

Forearm blood flow was measured by the technique of venous occlusion plethysmography using a Whitney (Whitney, 1953) mercury-in-silastic strain gauge. The left arm was supported comfortably at the wrist, approximately 15° above horizontal. Prior to flow determinations, a wrist band was tightened to suprasystolic pressures (~200 mm Hg) to exclude hand circulation. A second cuff, located just proximal to the elbow, was inflated to supradiastolic pressures (~60 to 70 mm Hg) for approximately eight to ten seconds for compression of the forearm veins. The strain gauge, placed at the level of greatest

forearm circumference, was used to measure changes in the volume of the forearm during venous occlusion. Following a series of six to seven blood flow measurements, the wrist band was released. Total occlusion time was approximately two to three minutes for each series of measurements. On each visit to the laboratory, two series of measurements took place, one at ambient pressure and one at the treatment pressure. Use of the arms for pedaling precluded blood flow measurements during exercise.

Forearm blood flow was calculated from the slope of a graph representing changes in forearm volume over time during occlusion (Greenfield, Whitney, & Mowbray, 1963). The last four measurements (of the series of six to seven measurements taken) were averaged to obtain the blood flow values that are reported. The strain gauge was calibrated while on the arm by measuring changes in electrical resistance in response to small degrees of known stretch.

In addition, a mercury-strain gauge was placed around the left calf at the position of greatest circumference. The change in calf circumference was monitored continuously throughout the entire test session. Blood flow *per se* was not measured in the legs; i.e., no venous occlusion was performed. Changes in calf circumference were monitored by the changes in electrical resistance on the plysthesmographic output, before and after application of the experimental pressure. An increase in leg circumference, registered by an increase in voltage, denoted an increase in the pooling of blood in the legs. Conversely, a decrease in leg circumference, registered by a decrease in voltage, indicated a decrease in blood volume in the legs.

Test Measures

Heart rate was recorded and monitored continuously via a data acquisition system (MacPac MP100, BioPac Systems, Goleta, California 93117) interfaced with a Macintosh computer. Blood flow measures were obtained at rest and beginning approximately 20 to 30 seconds following application of the experimental pressure. Systolic and diastolic blood pressures were measured using standard auscultatory techniques during the last minute of the initial five-minute rest and during the last minute of rest while under the influence of the experimental pressure. Use of the arms for cycling precluded blood pressure determinations during exercise. Mean arterial pressure (MAP), the average pressure over time, was calculated as follows:

$$MAP = DBP + 1/3(SBP-DBP)$$

where DBP is diastolic blood pressure and SBP is systolic blood pressure.

Gas exchange and ventilation data were collected and monitored continuously throughout each twenty-minute test session using a SensorMedics 2400 Metabolic Measurement Cart (Yorba Linda, California 92687). This cart analyzes and reports oxygen uptake as an average over a twenty-second collection period. Kinetic analysis was performed on data obtained during the transition from rest to exercise. A first-order model was used to calculate the time course of $\dot{V}O_2$ adjustment (Hughson & Morrissey, 1982; Hughson & Morrissey,

1983; Linnarsson, 1974) . The following monoexponential function was used:

$$f(t) = a\left(1 - e^{-\frac{(t-T_D)}{\tau}}\right)$$

where f(t) is $\dot{V}O_2$ at any given time (t), a is the amplitude of the response (or the asymptotic $\dot{V}O_2$ value), T_D is the time delay, and τ is the time constant. This first-order model is graphically illustrated in Figure 4. Actual computations were performed using *Mathematica* (Wolfram Research, 1993) an analytical software program for the Macintosh.

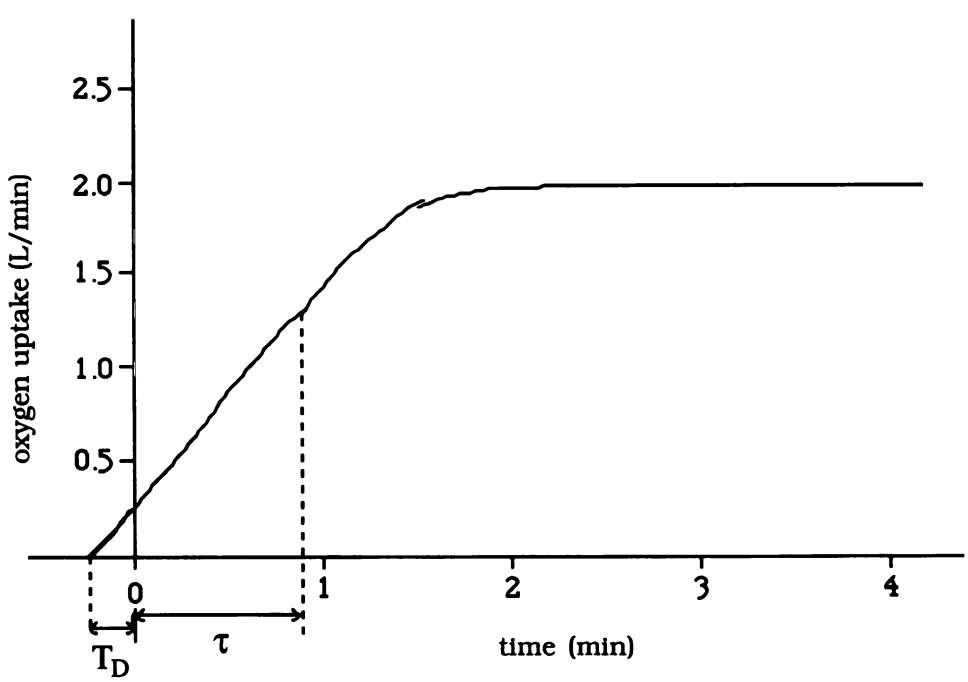


Figure 4. Graphical representation of the first-order kinetic model.

Both tau (τ) and the mean response time ($MRT=T_D + \tau$) were used to indicate the rate of adjustment of $\dot{V}_{O_2}$ toward steady state. For this first-order model, $\dot{V}_{O_2}$ reaches 63% of its amplitude at the time value of τ. The overall rate of change of the response is characterized by the MRT. Both these parameters (τ and MRT) are used as a means to quantitatively compare the time courses of $\dot{V}_{O_2}$ adjustment among experimental conditions (Hughson & Morrissey, 1982; Hughson & Morrissey, 1983; Linnarsson, 1974).

Blood samples were collected via a finger poke and tested for lactate prior to each test session and two minutes following the end of exercise.

Statistical Analysis

Each subject acted as his or her own control under all five treatment conditions. An analysis of variance for repeated measures was used to compare physiologic values obtained with the five experimental pressures. Tukey's *post hoc* method was utilized for comparisons with mean values at ambient pressure when significant differences were indicated by the ANOVA. The within-subject's error term was used for these analyses. An alpha of 0.05 was used for the level of statistical significance.

CHAPTER IV

RESULTS AND DISCUSSION

"Only peripheral vasoconstriction will restore blood pressure when it falls under conditions that limit ventricular filling pressure and prevent a rise in cardiac output."

L. Rowell, 1986

The content of this chapter is divided into four major sections. Subject characteristics are presented first. Next are the physiologic changes that occurred due to the transitions which took place initially from ambient pressure to each of the five experimental pressures with the subject at rest and then from rest to exercise while the experimental pressure was maintained. The third section deals with comparisons of physiologic parameters, including blood flows and oxygen uptake kinetics, measured across applications of the five experimental pressures. A discussion of the results as well as comparisons with related literature are presented in the final section. All results are expressed as mean $\pm$ s.d. unless otherwise noted.

Subject Characteristics

Five males and two females completed exercise bouts at each of the five randomly assigned lower body pressures. A review of medical histories revealed no prior respiratory or cardiac concerns. All

subjects expired at least 82% of their individual vital capacities in the first second of forced expiration (FEV_1), indicating that there were no respiratory problems at the time of data collection. Electrocardiograms appeared normal on all subjects. A summary of subject characteristics is presented in Table 1.

Table 1. Subject characteristics

ID	age	sex	height (cm)	weight (kg)	vital capacity (liters)	FEV_1 (liters)	FEV_1 % of VC
A	21	M	178.4	78.4	4.5	4.0	88.9
B	41	M	182.9	87.3	5.1	4.5	88.2
C	21	F	157.5	58.0	2.9	2.4	82.8
E	21	F	161.3	62.3	3.9	3.3	84.6
F	21	M	171.5	76.5	4.8	4.6	95.8
G	40	M	188.0	86.4	5.1	4.2	82.4
H	25	M	184.2	98.6	4.5	3.8	84.4
$\overline{X}$	27		174.8	78.2	4.4	3.8	86.7
s.d.	9		11.8	14.3	0.8	0.8	4.7

Physiologic Changes Due to Alterations in Lower Body Pressure and Exercise

On each of five visits to the laboratory, physiologic measurements were obtained for a total of ten minutes with the subject resting in a supine position. For the first five minutes, the subject was under the influence of ambient pressure. At the beginning of the sixth minute, one of five experimental pressures was applied (-40, -20, ambient, +20, and +40 mm Hg). This pressure was maintained for the next five minutes with the subject still at rest. At the beginning of the eleventh minute, submaximal supine arm exercise was begun (25 watts). Exercise was continued at a constant workload for the next ten minutes with the subject still under the influence of the experimental pressure. The results presented in this section summarize the physiologic changes that occurred from ambient to experimental pressures. In addition, changes in heart rate and oxygen consumption that occurred from rest to exercise during application of the experimental pressure are presented.

Changes in Circumference

Figure 5 shows typical plythesmographic outputs from mercury strain gauges placed on both the calf and the forearm. Following application of lower body negative pressure (see Figure 5a) blood volume was redistributed toward the lower extremities as seen by swelling of the calf (associated with a positve shift in voltage) and away from the upper body as seen by shrinking of the forearm (associated with a negative shift in voltage). This situation was reversed (see Figure 5b) during application of lower body positive pressure as seen

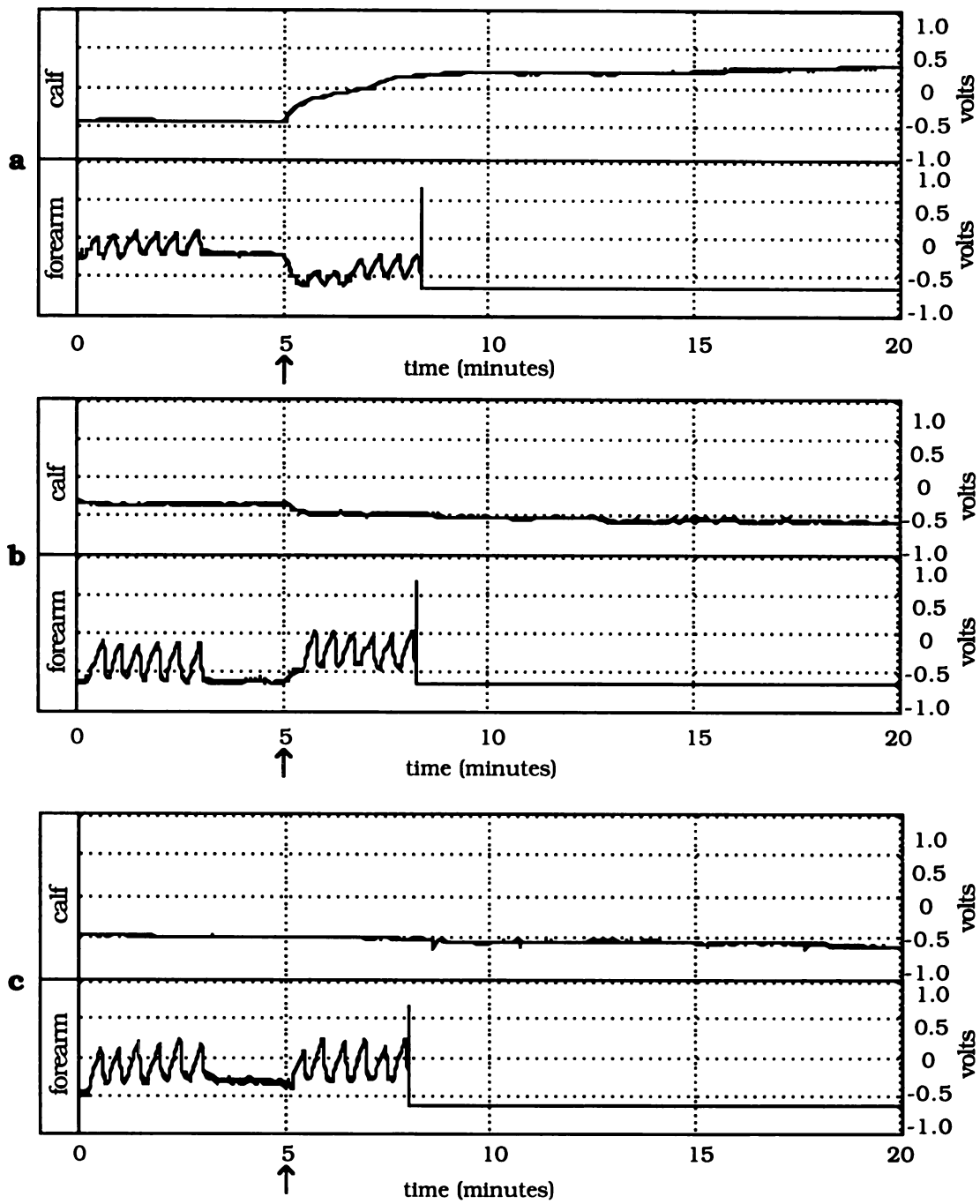


Figure 5. Typical plythesmographic outputs for the calf and forearm. Arrow represents application of pressure. a. Application of negative pressure. b. Application of positive pressure. c. Ambient-to-ambient pressure transition. Note loss of the signal from the forearm when the strain gauge was removed (at approximately eight minutes).

by shrinking of the calf and swelling of the forearm. As expected, there were essentially no changes in the circumference of either the calf or the forearm following the ambient-to-ambient pressure transition (see Figure 5c).

A summary of means and standard deviations for circumference changes (Figure 6) reveals a graded redistribution of blood volume in both the calf and the forearm at the five lower body pressures used. Average calf circumference changes were +5.10, +3.18, -0.27, -0.55, and -0.60 mm at -40, -20, ambient, +20, and +40 mm Hg respectively. Forearm circumference changes were of lesser magnitude and averaged -1.37, -0.80, -0.08, +0.51, and +1.11 mm at the same pressures.

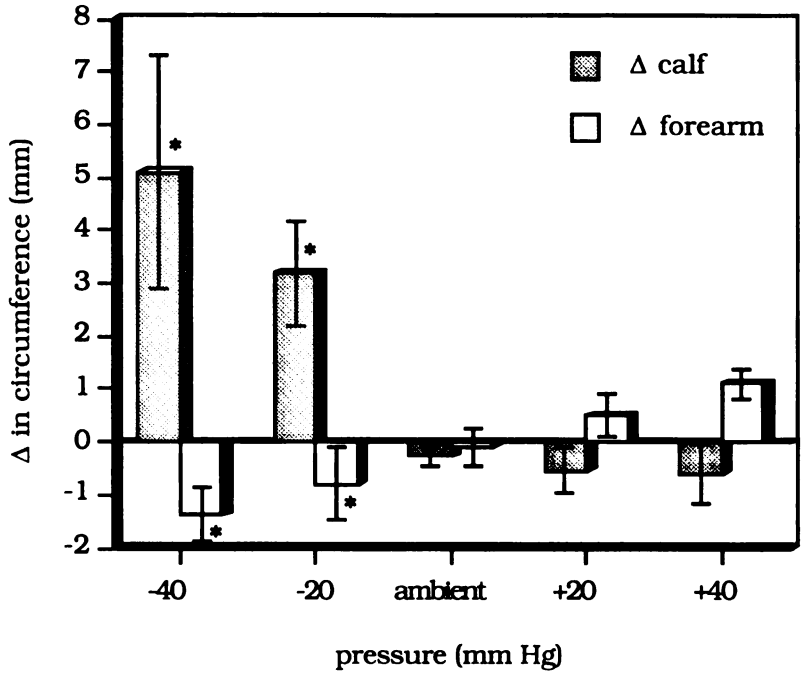


Figure 6. Changes in circumference. *=significantly different from change at the ambient-to-ambient pressure transition ($p<0.05$).

Changes in Heart Rate

Unlike the changes in circumferences, changes in heart rate appear to be graded only at lower body negative pressures (see Figure 7a). At these pressures, pooling of blood in the lower extremities reduces venous return to the heart and therefore stroke volume. In order to maintain cardiac output, heart rate increases. At -20 mm Hg, heart rate increased an average of 3 bpm or 5.4%. At -40 mm Hg, with more severe pooling of blood in the lower extremities, heart rate increased an average of 11 bpm or 19.7%. Transitions from ambient pressure to either ambient or positive (+20, or +40 mm Hg) pressures resulted in average heart rate decreases of less than 1 bpm or 1.5%.

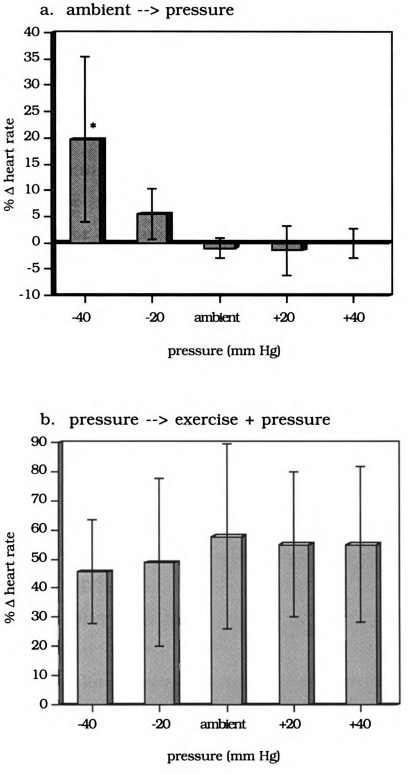


Figure 7. Changes in heart rate. *=significantly different from change at the ambient-to-ambient pressure transition ($p<0.05$).

Over the transition from rest to exercise, average heart rate increased at all pressures within a narrow range of 27 to 30 bpm or 46 to 58% (see Figure 7b). Since resting heart rates at -20 and -40 mm Hg already were increased above those at ambient pressure (see Figure 7a), the percentage increases in heart rate from rest to exercise during application of lower body negative pressure appear blunted (see Figure 7b). The resultant effect on steady state heart rates will be discussed in a subsequent section.

Changes in Oxygen Consumption

The changes in oxygen consumption following exposure of the lower body to various pressures are summarized in Figure 8a. Application of lower body negative pressure decreased the amount of oxygen consumed at rest by -9.0% at -20 mm Hg and by -11.0% at -40 mm Hg. Conversely, application of lower body positive pressure increased the amount of oxygen consumed at rest by 6.0% at +20 mm Hg and by 5.7% at +40 mm Hg. The effect of the ambient-to-ambient pressure transition was a trivial 1.9% increase.

Increases in oxygen consumption from rest to exercise averaged 502, 518, 473, 455, and 474 ml at -40, -20, ambient, +20, and +40 mm Hg respectively. The fact that oxygen consumption was already decreased at -40 and -20 mm Hg (see Figure 8a), coupled with the larger absolute increases in oxygen consumption at these same pressures, resulted in relatively larger percentage increases from rest to exercise at -40 mm Hg (+213%) and -20 mm Hg (+221%), than at ambient pressure (+174%), +20 mm Hg (+168%) or +40 mm Hg (+167%). These percentages are shown in Figure 8b.

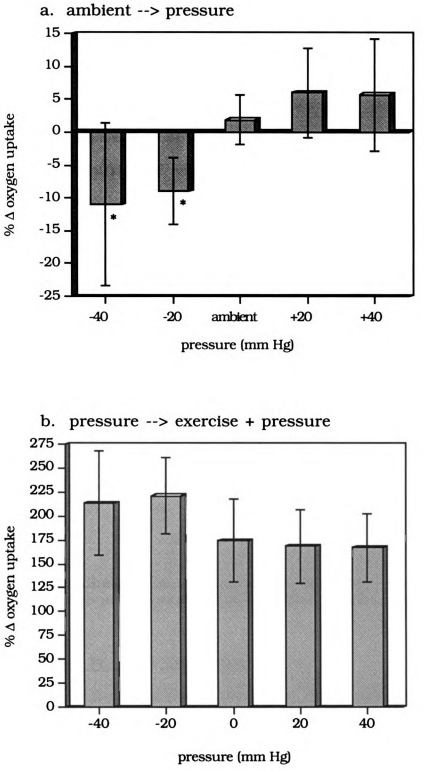


Figure 8. Changes in oxygen uptake. *=significantly different from change at the ambient-to-ambient pressure transition ($p<0.05$).

Changes in Mean Arterial Pressure

Mean arterial pressure dropped below that observed at lower body ambient pressure only at -40 mm Hg of lower body pressure (see Figure 9). At all other lower body pressures, mean arterial pressure increased above that at ambient lower body pressure, with the largest increase occurring at +40 mm Hg of lower body pressure (+9.1%).

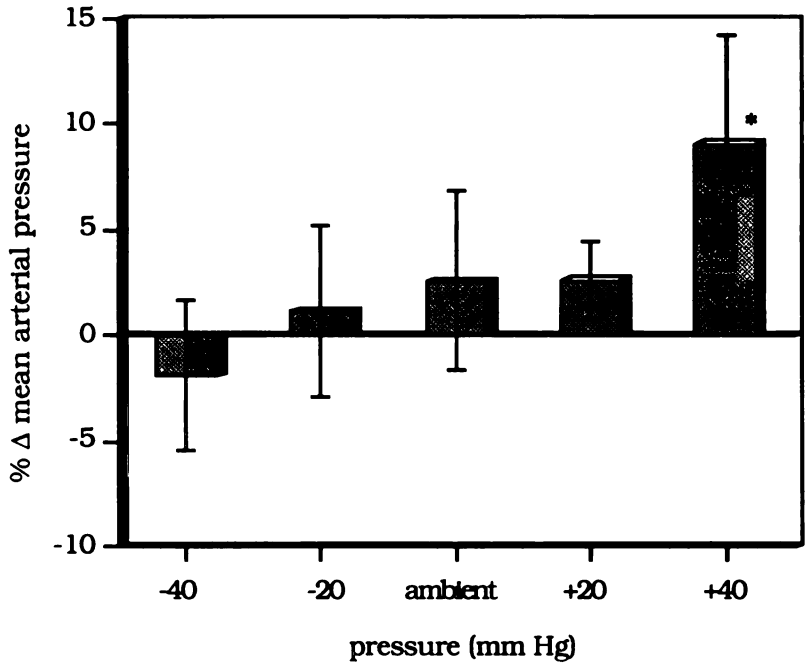


Figure 9. Changes in mean arterial pressure. *=significantly different from change at the ambient-to-ambient pressure transition ($p<0.05$).

Changes in Forearm Blood Flow

Table 2 shows that even before the experimental pressures were applied, rather large variations in resting forearm blood flows at ambient pressure occurred during the first five minutes across the five treatment conditions (i.e., 4.1 to 6.1 ml*100 $ml^{-1}*min^{-1}$). Absolute

blood flow decreased by a low of -0.1 ml*100 $ml^{-1}*min^{-1}$ at +20 mm Hg to a high of -1.3 ml*100 $ml^{-1}*min^{-1}$ at -40 mm Hg. This -1.3 ml*100 $ml^{-1}*min^{-1}$ decrease at -40 mm Hg coupled with the relatively low initial resting blood flow at ambient pressure (4.8 ml*100 $ml^{-1}*min^{-1}$) resulted in the largest percentage decrease (-23.4%) in blood flow during the transition from ambient pressure to experimental pressure. The next largest percentage decrease in blood flow occurred at -20 mm Hg (-21.5%). Figure 10 shows the percentage changes in blood flow at all five experimental pressures. The overall analysis of variance yielded a statistically significant F-value, but subsequent Tukey tests revealed no significant pairwise comparisons.

Table 2. Summary of forearm blood flow data. Blood flows are expressed as ml*100 $ml^{-1}*min^{-1}$ (mean±s.d.).

	lower body pressure (mm Hg)				
	-40	-20	ambient	+20	+40
baseline (ambient)	4.8±1.6	5.7±2.6	6.1±2.8	4.1±1.2	5.2±2.1
at pressure	3.5±0.7	4.5±2.2	5.6±2.2	4.0±1.3	4.6±1.4
Δ in blood flow	-1.3±1.2	-1.2±1.0	-0.5±0.8	-0.1±0.4	-0.6±1.3
% Δ in blood flow	-23.4±17.3	-21.5±13.2	-6.0±11.2	-3.5±12.2	-7.3±20.4

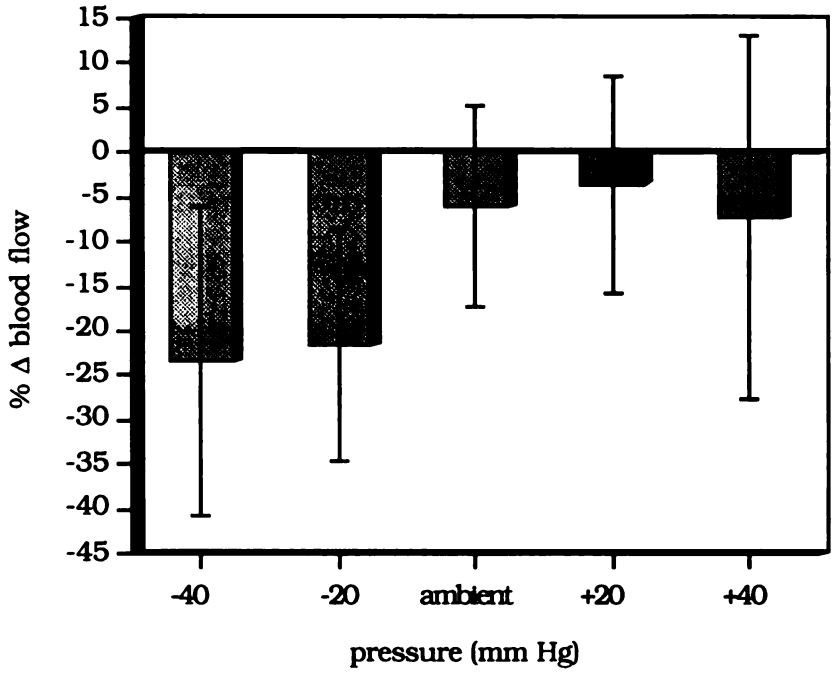


Figure 10. Changes in forearm blood flow. *=significantly different from the change at the ambient-to-ambient pressure transition ($p<0.05$).

Physiologic Comparisons Across the Five Experimental Pressures

The results presented in this section summarize and compare several physiologic parameters once the experimental pressures were achieved. Tukey's *post hoc* method was used to identify significant differences from mean values at ambient pressure when overall significance was indicated by an analysis of variance for repeated measures. Heart rate and oxygen uptake are compared across experimental pressures both at rest and during steady state exercise. Mean arterial pressure and blood flow are compared across experimental pressures at rest only, as these parameters could not be obtained during exercise. Lastly, the kinetic parameters, tau (τ) and

mean response time (MRT), are presented for the transition from rest to exercise.

Comparison of Heart Rates

Significant differences in heart rate across the five pressures were observed (see Figure 11) both at rest ($F<0.001$) and during steady state exercise ($F<0.001$). *Post-hoc* tests revealed that at rest, heart rate was significantly faster at -40 mm Hg (67 bpm) than at ambient pressure (55 bpm) ($P<0.05$). During steady state exercise, this difference persisted, as heart rate at -40 mm Hg (97 bpm) was elevated as compared to that at ambient pressure (83 bpm) ($p<0.05$).

Comparison of Oxygen Uptakes

Unlike heart rate, significant differences in oxygen uptake across the five pressures (see Figure 12) were seen at rest ($F<0.001$), but not during steady state exercise ($F=0.766$). At rest, oxygen uptake was significantly lower at both -40 mm Hg (244 ml) and -20 mm Hg (240 ml) than at ambient pressure (280 ml) ($p<0.05$). In spite of the low resting values at these lower body negative pressures, large percentage increases in oxygen uptake from rest to exercise at -40 and -20 mm Hg (see Figure 8b) eliminated any differences in steady state oxygen uptake among the five experimental pressures during exercise (see Figure 12b).

Whipp and Wasserman (1972) propose that leg exercise is occurring at a workload below anaerobic threshold if oxygen uptake during the third minute of exercise is not significantly different than oxygen uptake during the sixth minute of exercise. Because arm exercise, as used in the current investigation, has both a longer τ and

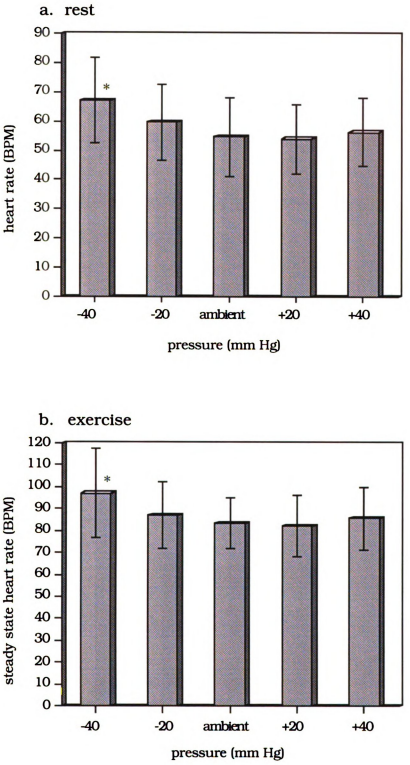


Figure 11. Heart rate during lower body pressure. *=significantly different from ambient ($p<0.05$).

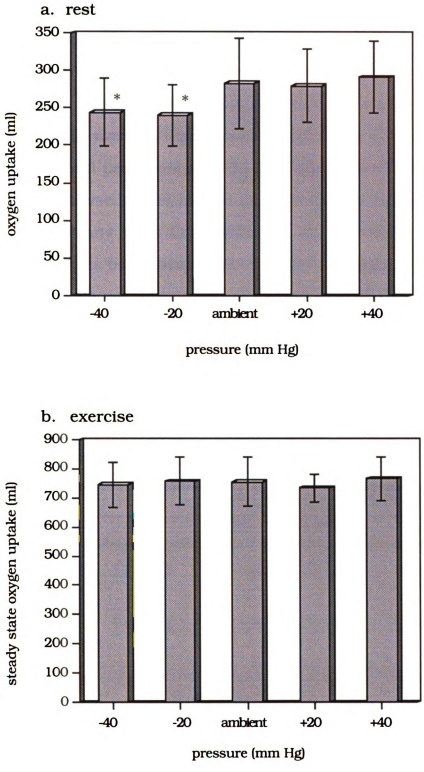


Figure 12. Oxygen uptake during lower body pressure. *=significantly different from ambient ($p<0.05$).

a longer MRT than does leg exercise, comparisons between values during the sixth and tenth minutes of exercise were used, as a conservative technique, to determine whether or not steady state was achieved. Paired t-tests of oxygen uptake during minute six and minute ten reveal no significant differences at any of the five experimental pressures used in this study (see Table 3). In addition, average unlysed blood lactate samples did not exceed 2 mM during exercise at any of the five pressures. This low value suggests that the workload was below anaerobic threshold although a statistical analysis of the lactate values was not conducted as a problem with the lactate analyzer caused approximately one-third of these values to be missing.

Table 3. Comparison of oxygen uptake during minutes six and ten. Oxygen uptakes are expressed as ml of oxygen (mean±s.d.).

	lower body pressure (mm Hg)				
	-40	-20	ambient	+20	+40
minute six	731±75	737±102	748±76	734±76	765±106
minute ten	748±86	771±99	754±101	734±60	762±65
significance	ns	ns	ns	ns	ns

Comparison of Mean Arterial Pressures

Application of lower body pressure significantly affected mean arterial pressure (F=0.020). *Post-hoc* comparisons revealed no significant differences from mean arterial pressure at ambient lower body pressure (see Figure 13). However, average mean arterial pressures at lower body pressures of both -40 mm Hg (93 mm Hg) and -20 mm Hg (92 mm Hg) were significantly lower ($p<0.05$) than was mean arterial pressure at the lower body pressure of +40 mm Hg (101 mm Hg), the lower body pressure at which mean arterial pressure increased by eight mm Hg over its value at ambient lower body pressure (see Figure 9).

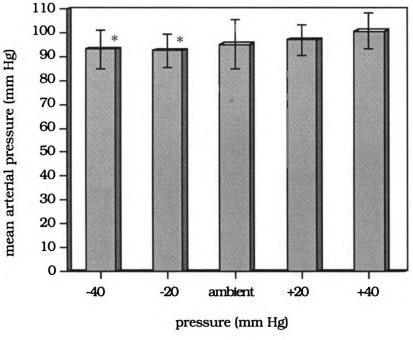


Figure 13. Mean arterial pressure during lower body pressure.
*=significantly different from +40 mm Hg ($p<0.05$).

Comparison of Forearm Blood Flows

No significant differences (P=0.098) were detected for average forearm blood flow values across the five experimental pressures. However, the power (0.559) for this analysis was relatively weak; and graphical representation of the data (see Figure 14) suggests a graded response in blood flow, at least at lower body negative pressures. The largest average blood flow was seen at ambient pressure (5.6 ml*100 $ml^{-1}*min^{-1}$). Blood flow dropped to 4.5 ml*100 $ml^{-1}*min^{-1}$ at -20 mm Hg and dropped even further to 3.5 ml*100 $ml^{-1}*min^{-1}$ at -40 mm Hg. Although not a graded response at positive pressures, blood flows were lower at both +20 and +40 mm Hg (4.0 and 4.6 ml*100 $ml^{-1}*min^{-1}$ respectively) than that seen at ambient pressure.

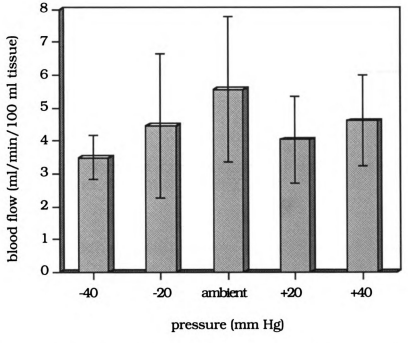


Figure 14. Blood flow during lower body pressure.

Comparison of Kinetic Parameters

Figure 15 shows a typical oxygen uptake graph for the transition from rest to mild arm exercise. Each point represents the average oxygen uptake during a 20-second collection period and is plotted at the midpoint of each period. Oxygen uptake at rest (time 0) was taken as the average oxygen uptake over the five minutes prior to exercise.

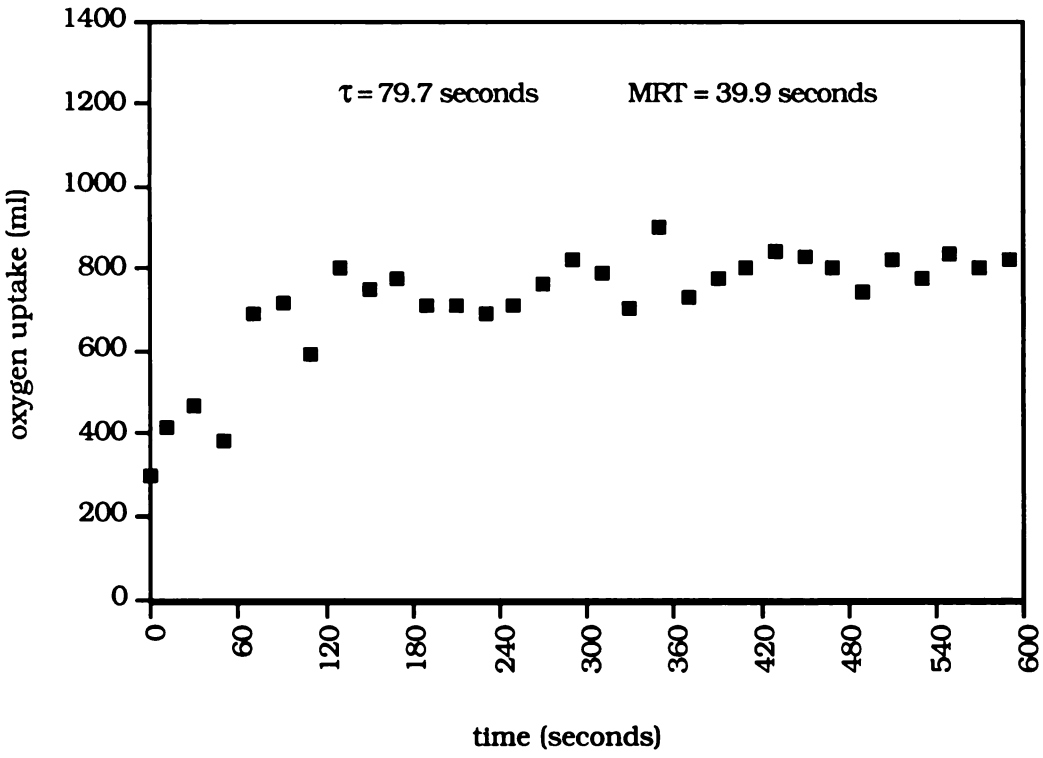
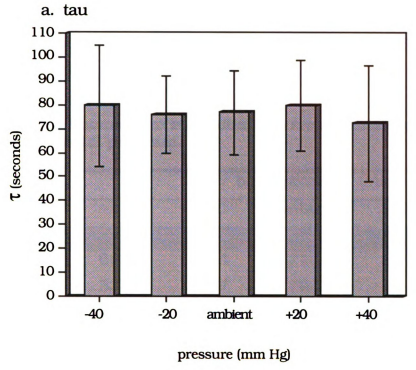


Figure 15. Typical oxygen uptake profile. Arm exercise began at 0 seconds and continued through 600 seconds.

The kinetic parameters, τ and MRT, are graphically shown at each lower body experimental pressure in Figure 16. No significant differences were detected across the experimental pressures for either τ ($F=0.867$) or MRT ($F=0.639$). Rather large variations, both



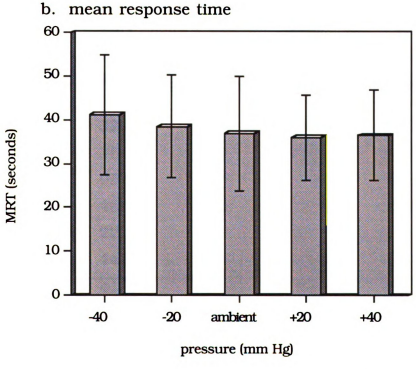


Figure 16. Kinetic analysis during lower body pressure.

within and between subjects (see Table 4), contributed to the low power for the ANOVAs run on τ (0.108) and on MRT (0.180).

Table 4. Summary of individual and mean kinetic parameters at each experimental pressure.

	lower body pressure (mm Hg)				
	-40	-20	ambient	+20	+40
τ (seconds)					
A	69.9	74.1	71.4	82.2	65.2
B	53.9	70.7	79.7	64.0	87.7
C	70.7	88.2	82.9	70.7	61.3
E	118.4	98.8	102.1	104.4	107.9
F	106.5	56.1	49.9	103.0	45.6
G	52.4	56.9	60.8	53.0	45.8
H	85.9	86.3	90.8	81.4	93.1
$\overline{X}$	79.7	75.8	76.8	79.8	72.4
s.d.	25.3	16.2	17.8	19.2	24.2
MRT (seconds)					
A	38.8	45.4	27.3	33.9	26.9
B	32.4	39.6	39.9	34.1	33.1
C	35.7	45.4	47.0	46.5	42.9
E	65.9	56.4	57.5	51.3	52.3
F	48.7	23.8	22.5	25.5	34.0
G	22.7	33.3	23.7	26.1	22.9
H	44.6	25.6	40.6	34.5	43.5
$\overline{X}$	41.2	38.5	36.9	36.0	36.5
s.d.	13.8	11.7	13.1	9.7	10.3

Because blood flow changes were not affected as expected at the experimental pressures used and the powers of both kinetic analyses were low, a further investigation of the relationships between blood flow and the kinetics parameters, τ and MRT, was undertaken. Multiple correlation was chosen as an approach to measure the strength of the association between blood flow, experimental pressure, and each of the kinetic parameters. Table 5 contains the results of these analyses.

Table 5. Multiple correlation results for kinetic parameters.

tau (seconds)		
multiple R = 0.28		
	T	probability
experimental pressure	-0.205	0.839
forearm blood flow	-1.608	0.118
MRT (seconds)		
multiple R = 0.45		
	T	probability
experimental pressure	-0.551	0.586
forearm blood flow	-2.655	0.012

Multiple correlation shows that experimental lower body pressure and forearm blood flow are more strongly correlated with mean response time (R=.45, $P<0.05$) than they are with τ (R=.28, P=0.26). In fact, the coefficient of determination (R^2) indicates that

the parameters experimental pressure and forearm blood flow together account for approximately 20% of the total variance in mean response time. Individually, the contribution by forearm blood flow was significant ($P<0.05$), but the contribution by experimental pressure was not (see Table 5). Only about 8% of the total variance in τ was explained by the combination of experimental pressure and forearm blood flow. Neither of these made a significant individual contribution.

Discussion

Alterations of the pressure surrounding the lower body resulted in characteristic redistributions of fluid within the body. Graded negative pressure (-20 and -40 mm Hg) resulted in graded translocation of blood volume from the upper to the lower body as seen by progressive swelling of the calves and shrinking of the forearms. Blood pools in the veins of the legs due to the increase in transmural pressure in these vessels. Graded positive pressure (+20 and +40 mm Hg) resulted in graded translocation of blood volume from the lower to the upper body as seen by progressive swelling of the forearms and shrinking of the calves. However, the magnitude of the translocation was much greater under the influence of negative pressure than it was under positive pressure. Although venous pressure was not measured in this study, other investigators have reported decreases in central venous pressure with application of lower body negative pressure (Bevegard, et al., 1977; Tripathi, Mack,

& Nadel, 1989) and only transient increases with application of lower body positive pressure (Bevegard, et al., 1977; Shi, et al., 1993).

As a result of the redistribution of blood volume when lower body pressure is altered, cardiovascular parameters are affected. At negative pressures, pooling of blood in the lower limbs reduces venous return which in turn reduces stroke volume (Bevegard, et al., 1977; Stevens & Lamb, 1965). In an attempt to maintain cardiac output, heart rate increases (Bevegard, et al., 1977; Lanne & Lundvall, 1992; Lightfoot, Claytor, Torok, Journell, & Fortney, 1989; Lundvall & Edfeldt, 1994; Rowell & Seals, 1990; Stevens & Lamb, 1965; Tripathi, et al., 1989; Vroman, Healy, & Kertzer, 1988). In the present study, this heart rate response appeared to be graded and reached statistical significance at -40 mm Hg.

At positive pressure, heart rate was unaffected. This observation agrees with the results obtained in other studies utilizing positive pressure (Bevegard, et al., 1977; Shi, et al., 1993). However, Shi et al. (1993) reported increases in both stroke volume and cardiac output at lower body pressures of +20 and +40 mm Hg, while Bevegard et al. (1977) did not at +40 mm Hg (the only pressure used in his study).

Pooling of blood in the lower limbs during LBNP also may affect oxygen uptake. Resting $\dot{V}O_2$ decreased significantly during the transitions from ambient pressure to both negative pressures. This decrease may be due to the fact that less blood was available to the upper body muscles, thereby decreasing the amount of oxygen extracted by them. No statistically significant increases were seen in $\dot{V}O_2$ at either of the positive pressures.

For adequate perfusion of vital organs, particularly the brain, blood pressure must be maintained when the body is stressed. At negative external pressures, mean arterial pressure was preserved at control levels in spite of the observed fluid redistribution and presumed fall in central venous pressure. This maintenance of blood pressure was observed by several other investigators utilizing lower body negative pressure, some even as low as -50 mm Hg (Bevegard, et al., 1977; Lundvall & Edfeldt, 1994; Rowell & Seals, 1990; Tripathi, et al., 1989).

Conversely, during lower body positive pressure ($\geq$+30 mm Hg) mean arterial pressure is elevated (Bevegard, et al., 1977; Shi, et al., 1993). In the present study, mean arterial pressure showed graded elevations at positive external pressures and reached a value at +40 mm Hg external pressure which was significantly higher than the values obtained at either of the external negative pressures, but not significantly different from the value obtained at external ambient pressure. Since the increase in stroke volume and cardiac output begins to level off at lower body positive pressures between +20 and +40 mm Hg, Shi et al. (1993) suggests that the increase in MAP at $\geq$+30 mm Hg is a result of activation of an intramuscular pressure-sensitive reflex.

The maintenance of blood pressure when blood is pooled in the lower extremities appears to be a result of two separate compensatory cardiovascular reflexes (Johnson, et al., 1974; Tripathi, et al., 1989; Zoller, Mark, Abboud, Schmid, & Heistad, 1972). At moderate levels of external negative pressure, blood pressure is maintained due to a

vasoconstrictor response. At more pronounced levels of external negative pressure, a cardioaccelerator response is activated via high-pressure baroreceptor stimulation.

During lower body negative pressure, vasoconstriction appears to be an early compensatory reflex that is triggered by unloading of low-pressure cardiopulmonary receptors at decreased filling pressures (Johnson, et al., 1974; Zoller, et al., 1972). This vasoconstriction is evidenced by the decreases in splanchnic (Johnson, et al., 1974) and forearm blood flows (Bevegard, et al., 1977; Essandoh, et al., 1986; Johnson, et al., 1974; Lundvall & Edfeldt, 1994; Tripathi, et al., 1989; Tripathi & Nadel, 1986; Zoller, et al., 1972). By comparison, during low-levels of lower body positive pressure, selective loading of the low-pressure cardiopulmonary receptors does not trigger withdrawal of sympathetic vasoconstriction of the forearm; and, as a result, there is no change in forearm blood flow (Shi, et al., 1993). However, forearm blood flow is significantly increased at high-levels of externally applied lower body positive pressure (Bevegard, et al., 1977; Shi, et al., 1993). Apparently, the cardiopulmonary receptors are more sensitive to unloading than to loading. The rapid vasoconstrictor reflex due to unloading probably provides a protective mechanism for humans when they change from supine to upright posture.

The statistically significant decreases (Bevegard, et al., 1977; Essandoh, et al., 1986; Johnson, et al., 1974; Lundvall & Edfeldt, 1994; Tripathi, et al., 1989; Tripathi & Nadel, 1986; Zoller, et al., 1972) and increases (Bevegard, et al., 1977; Shi, et al., 1993) seen in forearm blood flow with LBNP and LBPP, respectively, did not occur in

the present study. On any given visit to the laboratory, a single treatment pressure was applied to each subject. Therefore, baseline blood flow before the transition from ambient to treatment pressure could not be held constant and, in fact, varied widely across visits. This large variation in initial flow readings complicated the attempt to create graded levels of blood flow at the treatment pressures. In addition, relatively cool room temperatures (21-23° C) likely resulted in substantial baseline vasoconstrictor tone, leaving little room for further vasoconstriction. In spite of these considerations, blood flow did decrease an average of 21.5% and 23.4% at -20 and -40 mm Hg respectively. Although not statistically significant, a clear gradation was seen among forearm blood flow values at ambient pressure, -20 and -40 mm Hg (see Figure 14).

Much more difficult to explain is the lack of an increase in forearm blood flow at +40 mm Hg. Bevegard et al. (1977) propose that, in their study, the increase in forearm blood flow seen at +40 mm Hg probably reflected the net result of both thermo- and cardiopulmonary baroreceptors. Heating of the legs results in an increase in forearm blood flow, initially due to withdrawal of vasoconstrictor tone and later due to active vasodilation (Rowell, 1974a). Bevegard et al. (1977) did note an increase in chamber temperature of approximately 4-5° C during application of lower body positive pressure over a twenty-minute period. If thermoreceptors do contribute to the rise in forearm blood flow during LBPP as Bevegard et al. suggest, the lack of an increase in the present study may be due to the short period between application of the positive pressure and

the time when forearm blood flow was measured (approximately one minute). Forearm blood flow doesn't begin to rise until approximately five minutes following heating of the legs (Rowell, 1974a). In keeping with this finding, the increases in forearm blood flow reported earlier during LBPP were measured at least five minutes after application of the external pressure (Bevegard, et al., 1977; Shi, et al., 1993).

The results obtained on the kinetic parameters, τ and MRT, relate specifically to the research hypotheses underlying the conduct of this investigation. These results are somewhat difficult to interpret due to the lack of expected significant decreases in forearm blood flow at lower body negative pressures and corresponding increases in blood flow at lower body positive pressures. Although not significant, graded decreases in forearm blood flow at ambient pressure, -20 and -40 mm Hg paralleled graded increases in MRT at these externally applied lower body pressures (see Figures 14 and 16). The same graded pattern does not appear to hold for τ; however, the pattern may be masked by large within-treatment variations in this parameter.

At least one other investigator has shown faster kinetics (reported as MRT) using a procedure that was hypothesized to increase blood flow to working arm muscles (Hughson & Imman, 1986). In that study, circulatory occlusion of the legs, immediately following leg elevation in supine subjects, was used to increase central blood volume by decreasing venous volume in the legs. Although forearm blood flow was not measured, the author suggests that $\dot{V}O_2$ kinetics could not be increased unless the availability of oxygen also was increased through an increased blood flow.

The findings of the current and other investigations, including the fact that forearm blood flow and experimental pressure account for 20.0% of the total variance in mean response time (with a significant individual contribution by blood flow) justify further investigation into the possible role that oxygen delivery may play in limiting the immediate adjustment of $\dot{V}O_2$ at the onset of submaximal exercise.

CHAPTER V

SUMMARY, CONCLUSIONS AND RECOMMENDATIONS

"While the initial work of the muscles can be done on credit, adequate pay-as-you-go oxidation from atmospheric oxygen during continued exercise is dependent on the circulatory and ventilatory systems."

K. Wasserman et. al., 1967

Conflicting claims as to the mechanisms which limit the immediate adjustment of $\dot{V}O_2$ during the transition from rest to submaximal exercise have lead to much debate over the last decade. The physiologic mechanisms which may limit this rate of increase in $\dot{V}O_2$ can be grouped into two categories: (a) O_2 delivery via the cardiovascular system and (b) O_2 utilization by the working muscles.

To date, no experimental studies have been conducted in which O_2 utilization is altered acutely (e.g., by changing mitochondrial density, oxygen diffusion distances, etc.) to determine the effect on oxygen uptake kinetics. However, many investigators have altered O_2 delivery (e.g., by impairing cardiovascular function or restricting oxygen intake via hypoxia) in an attempt to solve this problem.

The purpose of the current study was to examine the effects of altered blood flow, hence oxygen delivery, on the rate of adjustment of $\dot{V}O_2$ at the onset of aerobic exercise. Lower body positive pressure (LBPP) was used in an attempt to increase blood flow to working arm

muscles. Lower body negative pressure was used in an attempt to decrease blood flow to working arm muscles. It was hypothesized that increasing blood flow to the arms would result in a faster rate of O_2 uptake adjustment and that decreasing blood flow to the arms would result in a slower rate of O_2 uptake adjustment.

Seven healthy subjects performed rest-to-exercise transitions on an arm ergometer under each of five experimental lower body pressures (-40, -20, +20, +40 mm Hg and ambient pressure). Each subject completed only one test session per day in random order. Each test session lasted twenty minutes and consisted of ten minutes of rest followed by ten minutes of exercise at a constant submaximal workload of 25 watts. During the first half of the rest period, the subjects were under the influence of ambient pressure; during the second half, they were under the influence of one of the five experimental lower body pressures. The pressure was maintained for the duration of the exercise bout.

Heart rate and measures of gas exchange were monitored continuously throughout the test session. Blood flows and blood pressures were measured during rest, both at ambient pressure and again at the experimental pressure. Use of the arms for cycling precluded these determinations during exercise. Blood lactate was measured prior to exercise and two minutes following completion of the exercise bout.

Data for each variable were plotted in two ways. First, as percentage changes that occurred due to the transitions from (a) ambient pressure to each of the five experimental pressures while

at rest and (b) from rest to exercise while the experimental pressure was maintained. Second, as data means at each of the five experimental pressures. An analysis of variance for repeated measures was used to compare physiological parameters across the five experimental conditions. Tukey's *post hoc* method was utilized for comparisons with mean values at ambient pressure when significant differences were indicated by the ANOVA.

Application of lower body negative pressure (-20 and -40 mm Hg) resulted in graded translocation of blood volume from the upper to the lower body. As expected, this translocation resulted in a graded increase in heart rate which reached statistical significance at -40 mm Hg. Oxygen consumption was significantly lower at both levels of negative pressure while at rest; however, no differences were detected across pressures during exercise. Mean arterial pressure and blood flow alterations were not statistically significant, although blood flow decreased an average of -21.5% and -23.4% at -20 and -40 mm Hg respectively.

Application of lower body positive pressure (+20 and +40 mm Hg) resulted in graded translocation of blood volume from the lower to the upper body; however, the magnitude of this change was much less than that seen during LBNP. Heart rate and oxygen consumption were unaffected by LBPP, while mean arterial pressure was greater than that seen at both -20 and -40 mm Hg. The expected increases in forearm blood flow during LBPP did not occur. In fact, decreases of -3.5% and -7.3% were detected at +20 and +40 mm Hg respectively.

No statistically significant differences were detected in either τ or MRT, the kinetic parameters used to assess the adjustment of $\dot{V}_{O_2}$ toward steady state during the transition from rest to submaximal exercise. However, large variations, both within and between subjects, contributed to low power for these analyses. Because the statistical power was low and the changes in blood flow were not as expected, multiple correlation was chosen as an alternative approach to analyzing the association between blood flow, experimental pressure, and each of the kinetic parameters.

Multiple correlation shows that experimental lower body pressure and forearm blood flow are more strongly correlated with mean response time ($R=.45$, $P<0.05$) than they are with τ ($R=.28$, $P=0.26$). In fact, the coefficient of determination (R^2) indicates that the parameters experimental pressure and forearm blood flow together account for approximately 20% of the total variance in mean response time. Individually, the contribution by forearm blood flow was significant ($P<0.05$), but the contribution by experimental pressure was not. Only about 8% of the total variance in τ was explained by the combination of experimental pressure and forearm blood flow. Neither of these made a significant individual contribution.

Conclusions

1. Experimental alteration of lower body pressure resulted in characteristic fluid shifts, increases in heart rate at lower body negative pressure, and increases in mean arterial pressure at lower

body positive pressure which were consistent with changes cited in the literature.

2. Application of lower body negative pressure is a reliable yet highly variable technique that can be used to decrease blood flow to the forearm.
3. Application of lower body positive pressure is an unreliable technique for increasing blood flow to the forearm. The increases seen by other investigators using LBPP may be related more to a thermal effect than to a loading of the cardiopulmonary receptors.
4. The observation that graded increases in mean response time parallel graded decreases in forearm blood flow together with the fact that forearm blood flow and experimental pressure account for 20.0% of the total variance in MRT (with a significant individual contribution by blood flow) suggest that oxygen delivery may be one factor which limits the immediate adjustment of oxygen uptake during the transition from rest to submaximal exercise.

Recommendations

The results of this investigation neither support nor refute the role of oxygen delivery in limiting the immediate adjustment of oxygen uptake during the transition from rest to submaximal exercise. However, the data suggest that further investigation in this area is warranted. The following recommendations are offered as points to be considered in future studies.

1. To reduce variability and increase the power of the statistical analyses, subjects who have similar baseline oxygen uptake kinetics

should be recruited. For example, trained subjects have faster $\dot{V}O_2$ kinetics than do untrained subjects. Therefore, recruiting all trained (or untrained) subjects would decrease the variability between subjects.

2. Although maximal work capacity was not assessed, anecdotal accounts suggest that, for some subjects, the 25-watt workload represented only a fraction of the workload that could have been maintained for the ten-minute exercise bout. A better approach would be to customize workloads so all subjects are working just below their individual anaerobic thresholds.
3. Since forearm blood flow is highly dependent on temperature, room temperature should be strictly controlled and maintained during all visits to the laboratory. In addition, cool room conditions should be avoided. Vasoconstriction due to lower body negative pressure is limited in forearm vessels already under the influence of substantial vasoconstrictor tone in the cold. Both room temperature and lower body chamber temperature should be monitored and analyzed in relationship to forearm blood flow.
4. Measures of blood flow were highly variable and could not be performed during exercise due to limitations of the technique of venous occlusion plethysmography. The use of magnetic resonance imaging (MRI) should be explored as an alternative method of measuring blood flow in these types of studies.
5. Alternative methods to increase forearm blood flow should be explored such as direct warming of the legs or application of LBPP for longer periods of time (> five minutes).

6. Alternative methods to increase oxygen delivery should be explored such as blood doping. The use of this technique to infuse red blood cells directly into the circulation would increase the oxygen carrying capacity of the blood, hence oxygen delivery to working muscles.
7. No experimental studies have been conducted in which oxygen utilization has been altered acutely in humans. Such a study would be a unique contribution to this area of research. The design of such an experiment would be highly challenging, however, as the possible factors affecting oxygen utilization are varied and difficult to modify (e.g., mitochondrial density, oxygen diffusion distances, the signal linking ATP demand to ATP supply, etc.).

APPENDICES

APPENDIX A

Health History Form

Health History

Identification

name:_____
first middle last

address:_____
street city state zip

social security #:_____ date of birth:_____

sex: ☐ female ☐ male height(in):_____ weight(lb.):_____
currently pregnant (females only): ☐ yes ☐ no

family physician:_____
name city state

Insurance Information

company:_____

policy #:_____

Emergency Notification

name:_____ phone #:_____

Respiratory System

yes	*no*	***In the past three years, have you had–***
☐	☐	frequent or persistent cough?
☐	☐	frequent or persistent wheezing?
☐	☐	frequent or severe shortness of breath while resting?
☐	☐	frequent or severe shortness of breath while exercising or working?
☐	☐	cough that produced a blood-tinged sputum?

Cardiovascular System

yes	*no*	***In the past three years, have you had–***
☐	☐	frequent or severe chest pain?
☐	☐	palpitations, irregular or skipped beats?
☐	☐	pain, pressure or tight feeling in chest which forced you to stop activity?
☐	☐	prolonged rapid heartbeat, even at rest?
☐	☐	heart murmur or "extra sound"?
☐	☐	heart valve problem, i.e., mitral valve prolapse?
☐	☐	recurrent swelling of feet and ankles?
☐	☐	recurrent calf pain when exercising or at rest?
☐	☐	shortness of breath while lying down, relieved upon sitting up?
☐	☐	painful redness or discoloration of the extremities, made worse by cold temperatures?

APPENDIX B

Informed Consent

Michigan State University
Alma College

Informed Consent

The experimental protocols and measurement procedures to be used in this exercise study have been explained to me. I agree to serve voluntarily as a subject in the research described. Participation involves returning to the laboratory five times over a period of approximately two weeks for one to two hours each visit. I understand that the research is being undertaken to further knowledge concerning the responses of individuals to exercise.

I understand that some physical discomfort may be experienced and that no beneficial effects are guaranteed. I further understand that there are potential risks involved in being exposed to lower body pressure changes. These risks include, but are not limited to, dizziness, sweating, nausea and/or fainting. I understand every reasonable effort will be made to minimize these risks and that trained personnel are available to deal with unusual situations that may arise.

I have had an opportunity to ask questions regarding the test and procedures to be used. I am free to ask additional questions of the investigators any time during the study; their home and office phone numbers have been provided to me. Furthermore, I have been informed that I am free to withdraw my consent and to discontinue my participation at any time.

I understand that group results may be used in scientific publications with my anonymity assured, that the data of individuals will be treated in strict confidence, and that my results will be made available to me upon my request.

I understand that if I am injured as a result of my participation in this research project, Alma College will provide emergency medical treatment. I further understand that if the injury is not caused by the negligence of Alma College, I am personally responsible for the expense of this emergency care and any other medical expenses incurred as a result of this injury.

_____	_____
subject signature	investigator signature
_____	_____
date	date

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