

To the Graduate Council:

I am submitting herewith a thesis written by Michael C. Hogan entitled "The Effect of Altering the Inspired Oxygen Fraction on Lactate Accumulation During Progressive Exercise." I have examined the final copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Zoology.

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THE EFFECT OF ALTERING THE INSPIRED OXYGEN
FRACTION ON LACTATE ACCUMULATION
DURING PROGRESSIVE EXERCISE

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ABSTRACT

These experiments were designed to investigate differences in lactate threshold and blood lactate accumulation during progressive bicycle ergometer exercise while breathing hypoxic, normoxic, or hyperoxic gas mixtures. Six subjects pedaled a stationary cycle ergometer to the point of exhaustion on three different occasions while breathing mixtures of 17%, 21%, or 60% O₂. After a 10-minute rest period, the subject rode for 3 minutes at a work rate of 60 W followed by 15 W increases every 3 minutes until exhaustion. Inspired and expired gas fractions, ventilation, heart rate, and arterialized venous lactate were measured. Oxygen uptake ($\dot{V}O_2$) and carbon dioxide output were calculated for the last minute of each work rate while lactate samples were drawn during the last 10 seconds. $\dot{V}O_2$ was not significantly different at any work rate among the three different conditions. $\dot{V}O_{2\text{ max}}$ did not differ significantly, but approached significance, among the three treatments. The rate of lactate accumulation was significantly greater during hypoxia as compared to either normoxia or hyperoxia. Lactate concentrations were significantly lower during hyperoxia and significantly higher during hypoxia when compared to normoxia. But when lactate levels were plotted against percent of $\dot{V}O_{2\text{ max}}$ for each treatment, no significant difference was found among treatments. A breaking point at which lactate increased above resting levels was determined. This occurred 15 W higher in hyperoxia than normoxia or hypoxia. Maximal lactate values at exhaustion were not significantly different among the three treatments.

Four subjects were able to work for a longer period of time during hyperoxic breathing. These results indicate that variations in lactate threshold and accumulation as reported in this study cannot be explained on the basis of differences in $\dot{V}O_2$. Differences in transient tissue hypoxia, excess pyruvate and other factors must be considered in an explanation of these data. This research also supports the hypothesis that decreases in lactate and H^+ accumulation during hyperoxic breathing result in the increases reported in performance times while breathing hyperoxic mixtures.

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CHAPTER I

INTRODUCTION

The role of lactate in the development of fatigue in exercising humans remains an unresolved and controversial area of research. During exercise in which there is an increase in muscle and blood lactate concentrations above resting levels, there are acid-base changes within the working tissue that may lead to the impairment of muscular performance. The elevation in blood lactate that is measured during exercise of this type is a result of the production by the working muscle, release into the blood by the muscle, distribution throughout the body compartments, and removal by some tissues. There is disagreement as to the role each of these processes has in lactate accumulation, but the end result in exercise of moderate to high intensity and of short duration is an elevation in the muscle and blood lactate concentrations.

The increase in lactate is part of the metabolic response of the working muscle to an elevated demand for energy. Chemically bound energy is transformed into mechanical work by the muscles when physical work is performed. The energy for work becomes available during the dephosphorylation of ATP, which can then be resynthesized either aerobically or anaerobically. ATP resynthesis can occur anaerobically when pyruvic acid formed during glycolysis is converted to lactic acid. Lactate, which is the ionization product of lactic acid, is formed during glycolysis so that the oxidation of NADH_2 can occur. This reconstitution of NADH_2 to NAD is necessary for ATP resynthesis to continue. The result of an

increased reliance on ATP resynthesis through glycolysis is an increase in lactate in the working cell. The lactate formed can then be oxidized or released from the muscle and into the blood. Lactate formation during exercise is thought to occur in principally two situations: at the start of exercise and until a steady state level is obtained, and with maximal work loads (Karlsson, 1971).

Investigators have identified a lactate threshold (or anaerobic threshold, onset of plasma lactate accumulation, aerobic threshold, early lactate threshold) as the point during progressive exercise when lactate measured in the blood starts to rise above resting levels. As the exercise becomes progressively more difficult, the muscle and blood lactate concentrations also increase up to a maximal point reached at exhaustion. Studies using varying levels of hypoxic or hyperoxic gases have demonstrated that lactate levels are elevated at the same work rate or oxygen uptake during hypoxia and that lactate levels are decreased at the same work rate or oxygen uptake during hyperoxia when compared to normoxia. These studies focused on the effect of the inspired PO_2 on the plasma lactate concentration during steady state work (Lundin and Strom, 1947) or near maximal work (Adams and Welch, 1980). A question exists, then, as to whether variations in PO_2 influence the time of appearance of lactate (lactate threshold) when the oxygen uptake is the same for different inspired gas mixtures. The first purpose of this study was to determine the lactate threshold during a progressive maximal work test while breathing hypoxic, normoxic, and hyperoxic gas mixtures.

After the initial appearance of lactate, Keul et al. (1967) showed that increases in lactate can shift the acid-base balance of the muscle

and blood, leading to a lowered pH. This increase in H^+ concentration has been postulated as one of the possible factors leading to muscle fatigue (Jones et al., 1977). Using hypoxic, normoxic, and hyperoxic gas mixtures as the inspired gas, Adams and Welch (1980) found that exhaustion was reached at the same lowered blood pH but at different times. They postulated that this could be due to the dissimilar rates of lactate accumulation in the steady state exercise studied, and that even though the rate of oxygen uptake in the three cases was the same, there may have been a difference in the rate of lactate formation as a result of the differences in PO_2 .

It has also been shown that performance times in hypoxia are decreased and that performance times in hyperoxia are increased, even though oxygen delivery is thought to be the same at equivalent work rates and oxygen uptakes (Hughes et al., 1968; Welch et al., 1977). Based on these observations, it is the second purpose of this study to answer the questions of whether during progressive exercise there are different rates of lactate accumulation and different total concentrations of lactate at similar work rates and O_2 uptakes while breathing different oxygen fractions. If it is found that lactate concentrations vary at the same oxygen uptakes, it will lend support to the hypothesis that alterations in the PO_2 have some effects on glycolysis that are not a result of changes in oxygen uptake. Although this study has not been primarily designed to investigate performance times to exhaustion, it will be possible to note differences in performance and relate these to the inspired gas fraction and rates of lactate accumulation.

CHAPTER II

REVIEW OF LITERATURE

Introduction

The review of literature will deal with the major research areas relevant to this study. These include: the dynamics of lactate accumulation and its control; the lactate threshold; and alterations in lactate, performance and other variables during exercise while breathing hypoxic, normoxic and hyperoxic gases.

Dynamics of Lactate Accumulation

Lactate formation. Muscle cells require energy in the form of ATP to perform work. As ATP is dephosphorylated to provide the necessary energy, it must be resynthesized or else work will halt. The phosphorylation of ADP occurs principally through glycolysis, the tricarboxylic acid cycle, and oxidative phosphorylation. Oxygen is necessary for the operation of the tricarboxylic acid cycle and oxidative phosphorylation, while glycolysis can function anaerobically. Carbohydrates and fats are the principal fuels used by the cells to form ATP. Since fats are only oxidized in the mitochondria, the breakdown of fats will always require oxygen. Carbohydrates are first broken down during glycolysis and converted to pyruvate, at which point at least two options are available. The pyruvate may be converted into acetyl-coenzyme A, which can enter the tricarboxylic acid cycle for further oxidation, or the pyruvate can

form lactate as an end product. The formation of lactate permits ATP resynthesis without the need for oxygen.

Whether these metabolic processes can proceed aerobically or anaerobically depends in part on the availability of oxygen in the cell. At low work rates, the energy metabolism is essentially aerobic since the oxygen supply is adequate. As the work becomes more intense, there is a shift from fatty acid oxidation to carbohydrate oxidation. At some critical intensity, the energy required by the working tissue becomes great enough that some of the pyruvate from the increased carbohydrate oxidation is transformed into lactate. At even higher intensities, more of the energy demands of the cell must be met through this process, resulting in greater amounts of lactic acid being produced (Karlsson, 1971).

The regulation of lactate production is a process which is not completely understood. But there is now good evidence that the phosphofructokinase reaction, which converts fructose 6-phosphate to fructose 1,6-diphosphate, is the major rate-limiting reaction in glycolysis. This enzyme is inhibited by high concentrations of ATP, citrate, long-chain fatty acids, hydrogen ion, and acetyl-coenzyme A; and it is stimulated by ADP or AMP (Lehninger, p. 425, 1975). Although phosphofructokinase possesses the characteristics necessary to be the main regulator of glycolysis, the enzyme phosphorylase, which converts glycogen to glucose, is also involved. Karlsson (1971) found that the increase in lactate concentrations begins only after the initial decrease in phosphagen (ATP + CP) stores within the working muscle. The enzyme phosphorylase might

be converted to its active form by the increase in cellular pH caused by the initial creatine phosphate depletion.

The enzyme lactate dehydrogenase (LDH) is another enzyme implicated in the control of lactate production and glycolysis. LDH, which catalyzes the reaction of pyruvic acid $\leftrightarrow$ lactic acid, exists in at least five different molecular forms, or isozymes, in higher animals. Each form of LDH has a different affinity for pyruvate and lactate. The H form favors a higher rate of pyruvate formation, whereas the M form favors a higher rate of lactate production. The rate at which the other forms of LDH will reduce pyruvate fall between the H and M forms. Gollnick et al. (1967) found that there was no change in muscle LDH activity during exercise and Karlsson (1971) revealed that the enzymatic activity of LDH does not limit the production of lactate. But the type of LDH isozyme found in the working muscle can influence the rate of lactate formation and oxidation (Sjodin, 1976).

Lactate release. After the lactic acid is formed in the cytoplasm of the working cell, it either diffuses or is transported across the cell membrane and into the blood. Lactate levels show a concentration gradient from muscle to blood, with muscle concentrations being greater. This gradient tends to be enhanced with increases in exercise intensity (Karlsson, 1971). Equal concentrations in the muscle and blood are usually obtained about 10 minutes after work stops. The highest concentration of lactate measured in the blood is usually obtained 4 to 10 minutes after the cessation of work (Margaria et al., 1963), but the highest concentration seen in the blood is not as great as the highest

concentration of lactate found in the working muscle (Karlsson, 1971). Karlsson (1971) and Jorfeldt et al. (1978) found an almost linear relationship between the highest blood lactate concentration and the muscle lactate concentration obtained immediately after cessation of work at several submaximal workloads. But Jorfeldt et al. (1978) found that at more severe workloads, a lactate efflux of about 4-5 mmole/min from the working muscle was maximal and that at these workloads the relationship between muscle and blood lactate was no longer linear. They interpreted their findings of a maximal level for the lactate release from the exercising muscles as evidence for some type of barrier to lactate movement within the muscle or at the cell membrane.

Distribution of lactate. The lactate released is then distributed throughout the body compartments. It has been found that the hydrogen ion release into the blood is equal to the lactate release during exercise (Sahlin, 1978). Since the production of lactic acid leads to this elevated H^+ concentration, the resulting acidity is compensated by the buffering systems of the body. The excess hydrogen ions produced by lactic acid formation are principally buffered by the bicarbonate system (Bouhuys et al., 1966). The excess CO_2 produced by the buffering of the bicarbonate system is released through ventilatory mechanisms.

Lactate removal. Lactate that has been produced will be removed by one of several processes. Lactate in the blood may be taken up by the liver and converted to glucose or glycogen (Lehninger, p. 432,

1975). In moderate, prolonged exercise, up to 50% of the lactate may be removed by hepatic-splanchnic tissues (Rowell et al., 1966). Cardiac muscle can use lactate as a fuel source (Carlsten et al., 1961). Resting muscle removes lactate during exercise (Poortmans et al., 1978; Jorfeldt, 1970), and lactate can be taken up (Stainsby and Welch, 1966) and oxidized by working skeletal muscle (Jorfeldt, 1970; Iseekutz et al., 1976; Minaire et al., 1975). Hermansen and Stensvold (1972) postulated from their experiments that the working skeletal muscle may be the main site for lactate removal during exercise. Edgerton et al. (1975) found that the rate of uptake of lactate into working skeletal muscle will depend on the concentration of arterial lactate as well as on the intra-muscular concentration of glycogen and lactate. Lactate that remains in the cell after exercise can be reconverted back to pyruvate and be used as a source of fuel (Lehninger, p. 768, 1975).

Lactate Accumulation During Exercise

The factors leading to lactate accumulation during exercise have been well studied, but there are still widely varying opinions on the causes of this phenomenon. Lactate production during exercise is thought to be a result of either pyruvate overflow (Huckabee, 1958) or some type of tissue hypoxia (Bang, 1936). At low to moderate work rates, there may be little lactate accumulation since the energy required can be made available through aerobic processes. At the higher work rates, however, the energy demands may be greater than the aerobic system's capacity, and some energy may be produced through the formation of lactate.

Karlsson (1971) and Jorfeldt et al. (1978) found that there was no marked increase in lactate concentration in the working muscle until work loads exceeded 50%-60% of maximal oxygen uptake ($\dot{V}O_2$ max). Other investigators that measured blood lactates are in agreement with these data (Costill, 1970; Senay et al., 1980; Davis et al., 1976), although trained athletes may be able to work at higher percentages of $\dot{V}O_2$ max before elevated concentrations of lactate are measured (Karlsson et al., 1972; Farrell et al., 1979). If the subject is performing steady state exercise, the concentration of blood lactate after an initial increase will depend on the intensity of the work. At moderate intensities (from 50%-70% of $\dot{V}O_2$ max), the lactate concentration increases for up to 20 minutes and then levels off or decreases. At the highest intensities (80%-100% of $\dot{V}O_2$ max) the blood lactate concentration will steadily increase up to exhaustion (Nagle et al., 1971; Karlsson and Saltin, 1970; Pendergast et al., 1979). In contrast to these results, Welch and Stainsby (1966) stimulated in situ dog skeletal muscle at various steady state work rates and found a transient increase in lactate production followed by a decrease in formation at all work rates studied. These results of a decrease in lactate production at $\dot{V}O_2$ max have not been duplicated in human studies. It should be noted that there are critical differences in animal studies that use small masses of muscle tissue and whole body, human studies.

The classical interpretation of lactate formation has been hypoxia of the muscle tissue. If this is true, there would be an increased dependence on anaerobic glycolysis for ATP resynthesis.

Tissue hypoxia could be a result of inadequate oxygen delivery to the tissues (Margaria, 1976; Ekblom et al., 1975; Horstman et al., 1976), or lactate formation could be caused by the muscle's inability to utilize a sufficient oxygen supply (Sahlin, 1978; Doll et al., 1968; Kaijser, 1970). At the moderate work loads where there is an initial increase in lactate concentration and then a leveling off or a drop, investigators have postulated that the lactate formation is due to an increased rate of glycolysis at the onset of work before the aerobic processes have had time to achieve a steady state rate (Kinderman et al., 1979; Bang, 1936; Hickson et al., 1978; Hughes et al., 1968; Wasserman et al., 1967; Wahren, 1977; McManus et al., 1974). A number of factors may contribute to the delay in the onset of aerobic metabolism at the beginning of this type of exercise. These probably include the time required for 1) the increase in ADP responsible for the acceleration of mitochondrial respiration, 2) the increase in cardiac output, and 3) the muscle blood flow elevation (Hickson et al., 1978). But Nagle et al. (1970) found in steady state exercise above 65% of maximal $\dot{V}O_2$ that lactate was elevated over the duration of the 30 to 60 minute runs. This study concluded that lactate is continuously produced throughout the exercise and not just at the beginning. This and other studies (Kindermann et al., 1979; Minaire et al., 1975) seems to favor a steady state of lactate formation balanced against a constant rate of lactate removal at these work rates. Above these work rates the lactate levels increase until exhaustion.

Other than transient tissue hypoxia, another factor influencing lactate formation during exercise may be the rate of pyruvate

production. If pyruvate formation occurs faster than it can be converted to acety-coenzyme A, then this elevated concentration of pyruvate could cause a mass action effect and lead to increased lactate formation, as suggested by Huckabee (1958). But Karlsson (1971) measured muscle pyruvate and lactate concentrations during exercise by using biopsy techniques and found an insignificant increase in pyruvate. He argued that "excess pyruvate" may be ignored as a significant cause of lactate formation. Other experiments also support these data (Linnarsson et al., 1974). However, the use of muscle biopsies to measure the concentrations of enzymes and reactions in working muscle may be questioned because the time required to obtain the muscle sample is lengthy in relation to the turnover rates of the enzymes or reactants. If in fact there is a pyruvate overflow during exercise, there have been no experimental results to confirm it.

Whether the lactate produced is due to actual tissue hypoxia beyond the time it takes the tissue to attain a steady state of oxygen uptake is still unclear. Jobsis and Stainsby (1968) showed that even during lactate production, measurements of the steady-state oxidation-reduction level of mitochondrial NAD in contracting mammalian skeletal muscle gives evidence of adequate oxygenation. Some investigators believe that at the highest work loads there is a real tissue hypoxia that leads to anaerobic glycolysis and lactate production (Hughes et al., 1968; Margaria, 1976), but other researchers have shown that even at maximal work rates there is no indication of tissue hypoxia (Doll et al., 1968). It is possible that the supply of oxygen is adequate at the lower work rates after a steady state is reached, but that there

are other factors that can lead to lactate production. The recruitment of muscle fibers during exercise has been implicated as one of these possible factors (Skinner and McLellan, 1980).

There are differences between the type I (slow twitch, oxidative) and type II (fast twitch, glycolytic) fibers that may account for some of the lactate formation at the sub-maximal work loads. The type I fibers are known to have a greater relative H-LDH activity, while there is almost three times as much M-LDH activity in the type II fibers (Thorstensson et al., 1977). Also, type II fibers have a lower capillary-fiber ratio, a lower mitochondrial concentration, and a lower rate of oxidative metabolism (Essen, 1978). Tesch (1980) found that intramuscular lactate levels were higher in the type II fiber after intense exercise. Based on studies investigating glycogen depletion patterns in the fiber types during progressive exercise, Edgerton et al. (1975) and others (Baldwin et al., 1977; Gollnick et al., 1975; Armstrong et al., 1975) found that type I fibers show a greater loss of glycogen at intensities of 30%-85% of $\dot{V}O_2$ max, and that type II fibers are recruited as the intensity or duration of work increases. Type II fibers, which appear to be more likely to become hypoxic, may be the major source of lactate. Thus, lactate production may depend on the muscle fiber composition of the subject and whether type I or type II fibers are being recruited.

The reliability of glycogen depletion experiments is limited, since other substrates are available to the working cell that can be used for energy (Essen, 1978). Essen also pointed out that the differences in glycogen content, oxidative capacity, and capillary-fiber

ratio between the fiber types may not be as great as once thought. With training, the fiber types may appear very similar. Data from other research led Saltin (1975) to believe, for reasons similar to those of Essen, that fiber recruitment patterns cannot adequately explain the lactate formation.

Another factor that has been associated with increases in lactate is the respiratory capacity of the muscle. It is well documented that after training, lactate levels at the same work rate will be decreased, even though the oxygen uptake will stay the same (Hickson et al., 1978; Karlsson et al., 1972; Ekblom et al., 1968). Saltin et al. (1976) used endurance training on just one leg and found significantly less lactate release in the trained leg even at the same oxygen uptake as the other leg. One argument to explain this would be that the increased mitochondrial density and enzymatic activity that occurs with training (Holloszy et al., 1975) increases the respiratory capacity of the muscle. It would then require an increased intensity of work to elevate the ADP concentration to the point of glycolytic stimulation (Ivy et al., 1980). Experiments by Karlsson et al. (1972), again using muscle biopsies, led them to speculate that the lowered lactate levels seen after training are caused by a decreased time to reach a steady-state of oxygen consumption or an increased oxidation of lactate by different tissues or organs.

The type of substrate that the muscle is using while it is performing work can also influence lactate production. At low intensities of work, free fatty acids are the predominant fuel source (Newsholme, 1977). Free fatty acid metabolism produces citrate, which

has already been shown to inhibit glycolysis. As the intensity of work increases, ADP will accumulate and stimulate glycolysis. Carbohydrates are therefore utilized to a greater extent and the capacity for lactate formation is increased. It has also been shown that lactate accumulation and the concurrent increase in metabolic acidosis will inhibit lipolysis--which will further increase the usage of carbohydrates (Issekutz et al., 1975). Jansson (1980) fed subjects a high fat diet for several days before the subjects exercised. She found a decrease in the amount of lactate formed at submaximal work loads. Thus, the availability of substrate, the rate at which it is utilized, and the amount of metabolic acidosis can all be possible factors that affect lactate accumulation.

Several hormones are known to influence substrate utilization and lactate production during exercise. Von Euler (1974) showed that during exercise, levels of epinephrine and norepinephrine increase. At the lower intensities, there is just a slight increase in catecholamines above resting levels. But as the intensity of work approaches maximal, there is a marked increase (Galbo et al., 1975). The concentration of plasma catecholamines increases with both the severity and duration of the exercise (Galbo et al., 1975). The increase in catecholamines, along with increases in growth hormone, glucagon, ACTH, and cortisol during exercise will increase free fatty acid mobilization and liver glucose output which may affect cellular metabolism (Galbo et al., 1977). The increase in the concentration of these hormones during exercise usually occurs around 60% of $\dot{V}O_2$ max, which means that the lactate increase that also occurs near that

percentage may negate the lipolytic activity of those hormones during progressive exercise. The hormonal regulation of fat and carbohydrate metabolism during exercise depends on the complex interaction between these factors. At this time, the interaction of these hormones during progressive exercise is still incompletely understood.

The Lactate Threshold

The lactate threshold (anaerobic threshold, onset of plasma lactate accumulation, early lactate threshold) is defined as the time or work rate during progressive exercise at which blood lactate starts to accumulate (Ivy et al., 1980; Wasserman et al., 1973). Wasserman and McIlroy (1964) were the first investigators to study this phenomenon in detail. They showed that during progressive exercise there is a point at which lactate levels become elevated above that which is found at rest. In a previous section it was shown that the lactate threshold usually occurs at work requiring 50%-60% of the maximum oxygen uptake (Wasserman et al., 1973), but in trained athletes it can occur at a higher percentage (Ivy et al., 1980). Most investigators use a progressive exercise test with the time at each work rate varying from 1 to 4 minutes (Skinner and McLellan, 1980). Except for the most intense work rates, a steady state of oxygen uptake and cardiovascular dynamics is usually achieved by the third minute in these types of tests (Hughes et al., 1968). While the lactate threshold deals with the onset of lactate accumulation, recent research (Davis et al., 1976; Wasserman et al., 1973; Reinhard et al., 1979) indicates that this threshold can be determined noninvasively by measuring ventilation

or the fraction of oxygen in the expired air. The reasoning is that as lactate spills into the blood, metabolic acidosis occurs and causes ventilatory compensation. So carbon dioxide output ($\dot{V}CO_2$) and ventilation ($\dot{V}E$) become non-linear relative to the increase in work rate or $\dot{V}O_2$ (due to the stimulation of ventilation by the excess CO_2 and H^+), and the fraction of expired oxygen (FE_{O_2}) drops. The ratio of $\dot{V}E/\dot{V}O_2$ has also been used to detect this threshold (Reinhard et al., 1979). Although the use of non-invasive measurements has been shown to be effective, there are some problems due to individual differences in rate of lactate transport from tissue to blood, buffering capacities, and the rate of elimination at different sites (Stamford et al., 1978). Also, there is not a good correlation between non-invasive lactate threshold measurements on the same individual on different days (Davis et al., 1976). For accurate measurements of the onset of blood lactate accumulation, it appears that direct blood sampling is the most reliable method.

Most of the possible factors that may influence the lactate threshold have been previously discussed, but their relationship to this phenomenon will be reviewed. With elevated plasma free fatty acids, the lactate threshold can be delayed (Ivy et al., 1979). This may be due to the inhibition of glycolysis by citrate. Recruitment patterns of muscle fibers might influence the lactate threshold, if in fact type II fibers have an increased propensity toward lactate production as discussed previously (Skinner and McLellan, 1980). It would be possible therefore, that as the intensity of work increases, there will be a point at which type II fibers must be recruited to

handle the extra energy requirements. This might initiate lactate formation. Ivy et al. (1980) found a significant correlation between the capacity of muscle homogenates to oxidize pyruvate (respiratory capacity) and the lactate threshold. They also found a significant correlation between percent slow-twitch fibers and lactate threshold. They concluded that a subject's respiratory capacity, which to a high degree depends on the mitochondrial content of the muscle, and percent slow-twitch fibers will determine the point at which lactate starts to appear in that subject's blood. Another possible factor in the onset of plasma lactate accumulation is that plasma levels of epinephrine and norepinephrine are also known to increase at about the lactate threshold (Haggendal et al., 1970). Silverberg et al. (1978) and Rizza et al. (1979) reported that at normal resting levels, catecholamines have little effect on metabolism, but at the increased concentrations measured during severe exercise, the catecholamines can influence metabolism. But since the concentration of catecholamines at the lactate threshold is still low, it is doubtful that any changes in metabolism that occur at the lactate threshold are due to changes in catecholamine concentrations (Rizza et al., 1979).

Many investigators (Hickson et al., 1978; Karlsson, 1971; Whipp and Wasserman, 1972; Pendergast et al., 1979; Linnarsson et al., 1974) believe that the lactate accumulation seen at submaximal work loads is due to the delayed time it takes for the muscle to reach an aerobic steady state. In fact a few investigators have found significant correlations between the oxygen deficit and lactate production (Gautier et al., 1978; Karlsson et al., 1972; Wasserman et al., 1967;

Linnarsson et al., 1974). Saiki et al. (1967) concluded from their research that at submaximal work rates there was no lactate production after a steady-state of O_2 uptake was achieved. But Nagle et al. (1970) showed that blood lactate levels were constant or increasing over time at work loads above 65% $\dot{V}O_2$ max. This implies that the lactate production continues throughout exercise and is not just due to a transient hypoxia at the initiation of work. How this possibility of transient hypoxia of the muscle tissue is related to the lactate threshold is uncertain. During progressive tests, the appearance of lactate in the blood of many subjects will occur at a specific work rate or percent of maximum oxygen uptake (Wasserman et al., 1973). If the lactate produced is due to the delayed time to reach steady state, it is unclear as to why the production occurs at that point, and not some earlier or later time. It would appear that the balance between lactate formation and removal within the muscle may be significant (Karlsson et al., 1972), but this relationship has not been adequately investigated.

In conclusion, it appears that substrate availability, recruitment of fiber types, respiratory capacity of the muscle, transient oxygen deficiency, production-removal rates and hormonal changes may all be possible factors in determining the lactate threshold. Several investigators have interpreted their experimental results to show that hypoxia due to insufficient oxygen delivery or utilization (Margaria, 1976; Ekblom et al., 1975; Wasserman et al., 1973) may determine the point at which lactate starts to accumulate in the muscle. But this has not been shown conclusively.

Variations in Lactate Accumulation and Performance Due to Alterations in the Inspired Oxygen Fraction

Experiments varying the inspired oxygen fraction have been used for many years to investigate several different physiological processes. Control of ventilation, acid-base changes, cardiovascular dynamics, metabolism, and changes in muscular performance have all been studied using hypoxia and hyperoxia. In exercise studies, hypoxia and hyperoxia have been classically used to decrease or increase the amount of oxygen that reaches the working muscle (Lundin and Strom, 1946; Bason et al., 1971; Ekblom et al., 1975; Asmussen et al., 1947). However, recent evidence indicates that oxygen delivery to the working muscle in hypoxia, normoxia or hyperoxia is constant at submaximal work rates (Hughes et al., 1968; Welch et al., 1977), although there have been exceptions to this observation (Barclay et al., 1979).

The effects of hypoxia and hyperoxia on the body during exercise are well documented. It is known that when the inspired oxygen fraction is less than normal, there is an increase (compared to normoxic conditions) in the following factors during exercise at the same work rate or oxygen uptake: ventilation (Hermansen and Saltin, 1967; McManus et al., 1974), heart rate (Lundin and Strom, 1947; Fagraeus et al., 1973), vaso-dilation (Guyton et al., 1964), lactate levels in the muscle and blood (Linnarsson et al., 1974; Hughes et al., 1968), respiratory quotient (Adams and Welch, 1980), and blood H^+ (Adams and Welch, 1980; Jones et al., 1972; Gladden and Welch, 1978). Hyperoxia is known to decrease (compared to normoxic conditions) the following factors

during exercise: ventilation (Gautier et al., 1978; Fagraeus et al., 1973), heart rate (Hughes et al., 1968; Welch et al., 1974), vasodilation (Cassuto and Farhi, 1979), lactate levels in the muscle and blood (Linnarsson et al., 1974; Hughes et al., 1968), and blood H⁺ (Adams and Welch, 1980). Performance time in maximal exercise is increased with hyperoxia (Adams and Welch, 1980; Wilson and Welch, 1975; Fagraeus et al., 1973) and decreased with hypoxia (Bason et al., 1971; Hughes et al., 1968; Ekblom et al., 1975). Oxygen uptake in hypoxia has been shown to be increased (Jones et al., 1972), equal to (Lundin and Strom, 1947; Hughes et al., 1968; Adams and Welch, 1980; Bason et al., 1971; McManus et al., 1974) or less (Ekblom et al., 1975; Fagraeus et al., 1973) than oxygen uptakes in normoxia at similar work rates. Oxygen uptake during hyperoxic breathing has been shown to be higher than (Welch et al., 1974; Ekblom et al., 1975; Linnarsson et al., 1974; Wilson et al., 1975; Fagraeus et al., 1973) or equal to (Stanek et al., 1979; Gautier et al., 1978; Hughes et al., 1968; Adams and Welch, 1980; Welch et al., 1977; Cassuto and Farhi, 1979; Wilson et al., 1975) oxygen uptakes in normoxia at equivalent work rates. It is believed by some investigators that oxygen uptakes are similar at the same work rate in the three conditions (Adams and Welch, 1980; Hughes et al., 1968), although exercise with a tracheal P_O₂ below 120 mm Hg will limit the maximal oxygen uptake (Hansen et al., 1967).

The question arises as to the cause of the difference in lactate concentrations seen in muscle and plasma during hypoxic, normoxic and hyperoxic conditions if the oxygen uptake is the same. If in fact the oxygen delivery to the working tissue is similar at equivalent oxygen

uptakes, then the lactate differences cannot be due to increased or decreased oxygen availability at the working tissue. Several investigators have found that the time it takes for the exercising muscle to reach a steady state of oxygen uptake after the onset of work is changed when the fraction of inspired oxygen (FI_{O_2}) is varied (Gautier et al., 1978; Hughes et al., 1968; McManus et al., 1974; Linnarsson et al., 1974). When breathing decreased oxygen concentrations, they found that there is an increase in the time required for the working tissue to reach a steady state, while increases in the inspired oxygen fraction lead to a decreased time for the muscle to reach a steady state of oxygen uptake. Thus these investigators postulated that the differences seen in lactate concentrations at the same oxygen uptake in hypoxia, normoxia and hyperoxia are due to dissimilar lag times in mobilizing the oxidative system in the working cell.

In a review article, Skinner and McLellan (1980) suggest that another possible factor that could lead to differences in the lactate concentration at similar oxygen uptakes is the fuel being metabolized by the working muscle. Issekutz et al. (1965) found that lactic acid inhibits free fatty acid release and oxidation, and that utilization of free fatty acids will inhibit glycolysis. Therefore an increased usage of free fatty acids can lead to a lowered lactate production. However, if glycogen metabolism is emphasized, there may be an increased lactate concentration and an inhibition of free fatty acid utilization. Although there are not many studies dealing with the balance of these factors in hypoxia and hyperoxia, it appears from a

study by Jones et al. (1972) that in hypoxia there is an increased mobilization and utilization of free fatty acids and an increased R concurrent with the increased lactate levels. These results seem contradictory.

The decreased R seen in hyperoxia (Gautier et al., 1978; Hughes et al., 1968; Adams and Welch, 1980; Welch et al., 1974; Wilson et al., 1975) might indicate an increased metabolism of free fatty acids, which could decrease lactate concentrations, but this has not been shown. The decrease in R seen during hyperoxia might also be due to a decreased elimination of CO_2 (Gautier et al., 1978; Welch et al., 1974), although Wilson and Welch (1975) argued against this possibility. Linnarsson et al. (1974) showed that glycogen depletion during hypoxia, normoxia and hyperoxia is the same, which suggests that fuel utilization is similar. The varied results of these experiments indicate the lack of understanding at the present time of metabolism during hypoxia and hyperoxia.

Ventilation while breathing hypoxic gas mixtures is increased above normoxic values and ventilation is decreased below normoxic levels while breathing hyperoxic mixtures. The possibility exists that the decreased cost of breathing in hyperoxia might lead to lowered lactate levels, while the increased cost of breathing in hypoxia might lead to elevated lactate levels. This might occur due to lactate production in the respiratory muscles. Hughes et al. (1968) found a close relationship between blood lactate levels and the rate of ventilation--which would seem to support this possibility, but Linnarsson et al. (1974), measuring muscle lactate levels, found

different lactate concentrations in the working muscle during the three gas conditions. This indicates that it is the muscle itself that is responsible for varied lactate levels and that the differences are not due to changes in the cost of breathing, except for possibly some variation at the highest work levels.

The Pasteur effect, which is a decreased rate of glycolysis when oxygenation increases, might be a factor in the altered rates of lactate formation seen with the various gas mixtures (Welch et al., 1974). Even though the oxygen delivery to the working muscle may be similar in hyperoxic and normoxic breathing conditions as a result of cardiovascular adaptations, there may be an increased oxygen pressure within the working tissue during hyperoxia that would slow glycolysis and lactate formation. While breathing hypoxic mixtures the opposite effect may occur. Rapoport et al. (1976) showed that when erythrocytes were taken from the oxygenated state to the deoxygenated state at a constant intracellular pH, glycolysis was increased by 26%. They postulated that the regulation of glycolysis when the PO_2 is changed is through the enzyme phosphofructokinase. This effect has not been shown in working skeletal muscle.

There have not been any investigations that have shown changes in the lactate threshold due to alterations in the inspired oxygen fraction. Most studies that utilize hypoxic or hyperoxic gas mixtures use steady state or maximal work. The few studies that have investigated lactate accumulation during progressive work tests have not been designed to note changes in the lactate threshold. Those that do report results show that in hypoxia, as compared to normoxia,

the threshold appears to occur at a lower oxygen uptake (but at the same percent of maximal oxygen uptake) (Hermansson and Saltin, 1967). Hughes et al. (1968) investigated the effects of hypoxia, normoxia and hyperoxia on exercise during progressive work. Their results were inconclusive because of the large work rate increments and long time periods at each work rate.

In conclusion, the results of research and the interpretations of investigators in this area are contradictory and there are still questions that need to be resolved. All of the factors outlined in this review need clarification in terms of what role they may play in the onset of lactate accumulation and its progressive rise. Whether some factors are more important than others or whether there is a complex interaction of factors needs to be determined. It is the purpose of this research, then, to plot lactate accumulation during progressive exercise under different oxygen concentrations in the inspired air to investigate the role of FI_{O_2} in these processes.

CHAPTER III

METHODS

Subjects

Six healthy male volunteer subjects participated in this study. All subjects were in good physical condition although none was trained as a cyclist. The subjects were all accustomed to the laboratory surroundings and all were comfortable with bicycle ergometer riding. All subjects signed informed consent forms before participating in the study. Their physical characteristics are presented in the Appendix, Table 1.

Preliminary Experimental Protocol

Each subject, after becoming accustomed to bicycle ergometer riding, performed a preliminary maximal oxygen uptake ($\dot{V}O_2$ max) test. This test was a continuous progressive test with heart rate, ventilation, FEO_2 , and $FECO_2$ monitored. A constant pedal frequency of 60 revolutions per minute was maintained until exhaustion. $\dot{V}O_2$ and $\dot{V}CO_2$ were calculated from gas samples of expired air that were collected during the test. The subject's highest calculated oxygen uptake was used as his $\dot{V}O_2$ max.

Gas Mixtures

Three gas mixtures were breathed by the subjects in this study. They were normoxia (21% oxygen--79% nitrogen), hypoxia (17% oxygen--83% nitrogen), and hyperoxia (60% oxygen--40% nitrogen). The gases

were mixed from commercially available compressed gas cylinders of 100% oxygen and 100% nitrogen. The composition of the mixtures was determined by electronic gas analyzers calibrated within the range of the gas mixture. The calibration tanks used to standardize the gas analyzers had their O_2 , CO_2 , and N_2 fractions measured using the Scholander apparatus. The tanks that were mixed for the tests were sampled periodically and analyzed by the Scholander method to ensure that the electronically recorded values were accurate.

There were three experiments for each subject, and each experiment varied only in the inspired oxygen fraction. The subject was kept unaware of which gas was being breathed. The six possible orders in which the mixed gases were presented to the subjects were assigned randomly so that an ordering effect could be minimized.

Blood Sampling Procedure

The subject rested in a supine position as a Deseret minicath-PRN catheter was inserted into a superficial vein on the back of the hand for blood sampling. A heparin-saline solution (120U/ml) was used to keep the catheter clear. After the catheter was taped into place, a 21 gauge needle was placed into the catheter reservoir so that blood flowing through the catheter and out the needle bore could be collected easily. Periodic flushing of the catheter with the sterile heparin-saline solution during the experiment ensured a steady flow of blood. Approximately 50 ml of blood was lost by the subject in each experiment. Blood samples were taken during the last 5 seconds of each work load by allowing the blood dripping out of the needle bore to

collect in a spot plate that had been coated with 4% NaFl to prevent coagulation and to stop glycolysis. Each blood sample was analyzed for lactate by a fluorometric modification of the method of Gutman and Wahlefeld (1974).

Experimental Protocol

All six subjects performed the three experiments in an identical manner except for the established order of the inspired oxygen fraction. The first test was performed at least 1 week after the subject's maximal oxygen uptake test and each test thereafter was conducted at 1 week intervals. This was done in order to minimize any possible training effects. Each test was structured in the same manner so that the subject was unaware of which mixture was being used. The subject reported at least 3 hours postprandial. Sleeping and training habits were kept uniform.

The subject reported to the lab, changed into gym clothes and lay down for 10 minutes. A heating pad was used to warm the hand to be catheterized. The catheterization was followed by another 5 to 10 minute rest period, at which time ECG electrodes were placed on the subject's chest for monitoring heart rate. The subject then sat on the ergometer and began breathing that day's gas mixture through a low resistance Daniels valve.

The gas first flowed through a humidifying chamber to moisten and warm the gas. From the humidifying chamber the saturated gas entered a Collins 120-liter spirometer which acted as a reservoir. From the spirometer the gas passed through a Parkinson-Cowan CD4 gas

meter and into the subject's mouthpiece. The expired gas then traveled from the subject's mouthpiece through a 44 cm length of tubing and into a 5 liter mixing chamber. The expired gas was sampled from the mixing chamber during the last minute of each work load. This was done by attaching a 1 m length of 6 mm-ID tubing, with a moisture absorbing unit attached, from the mixing chamber to an Applied Electrochemistry S-3A O₂ analyzer and a Beckman LB-2 CO₂ analyzer. Expired gas fractions of O₂ and CO₂ were read at 15 second intervals during the last minute of each work rate and were recorded by hand. The analyzers were calibrated during the experiment at 3 minute intervals with known gases. Before each test the entire system was flushed with that day's gas.

After the subject breathed that day's gas for 10 minutes, he started to pedal at 60 revolutions per minute. The subject rode at each work load for 3 minutes thereafter. The order of work loads was as follows: 1.0 kg, 1.5 kg, 1.75 kg, 2.0 kg, increasing by 0.25 kg every 3 minutes until the subject was exhausted. Exhaustion was defined as the point at which the subject could not continue for another 3 minute work load at the designated pedal frequency. Since each subject was accustomed to exhaustive exercise, there was little chance of having a subject quit before exhaustion. After the subject had stopped, he was allowed to remove his mouthpiece and sit quietly on the bicycle ergometer for 6 to 8 minutes.

Blood samples were taken during the last 5 seconds of rest and during the last 5 seconds of each 3 minute work load. Samples were also obtained 2, 4, and 6 minutes after exercise. After the sample

had been collected and mixed in the spot plate, a 50 μ l portion was mixed into 2 ml of 7% perchloric acid not more than 2 minutes after the sample was obtained. Blood sampled from arterialized venous blood in this manner accurately reflects arterial values (Forster et al., 1972).

Ventilation was recorded continuously by an ink writing oscillograph from a potentiometer on the Parkinson-Cowan CD4 gas meter. Heart rate from a Narco-Biosystems biotachometer BT 1200 was also recorded on the oscillograph. Calibration of the heart rate was performed several times during each test.

Occasionally gas samples were drawn into 50 ml lubricated syringes from the mixing chamber during the last minute of work to compare with the electronically recorded values. Meteorological balloons were used to collect gas samples during the last minute of some work loads to compare with the oxygen uptakes calculated by the methods used in this study.

Description of the Measurements

Expired gas fractions sampled from the mixing chamber were recorded from the electronic analyzers. $\dot{V}O_2$ and $\dot{V}CO_2$ were calculated by using the values recorded from the electronic analyzers. Adams and Welch (1980) found that $\dot{V}O_2$ and $\dot{V}CO_2$ calculated in this manner are accurate. The formula (Otis, 1964) used to calculate oxygen consumption was:

$$\dot{V}O_2 = \dot{V}_{ISTPD} (FIO_2 - FE O_2 \cdot FIN_2 / FEN_2)$$

Carbon dioxide measurements were checked in the same manner as the oxygen measurements. The formula (Otis, 1964) used to calculate carbon dioxide output was:

$$\dot{V}CO_2 = \dot{V}I_{STPD} (F_{IN_2}/F_{EN_2} \cdot F_{ECO_2} - F_{ICO_2})$$

Statistical Analysis

The means across subjects for oxygen uptake, lactate, heart rate and ventilation were determined for each work rate for each of the three gas treatments. Also the mean maximum value for each of these variables was calculated for each of the three treatments. To determine whether oxygen uptake varied among the three treatments, covariance analysis was used to analyze oxygen uptake over all work rates. This analysis was used after the test for parallel slopes requirement was met. A two-way ANOVA for repeated measures was used to analyze lactate, ventilation, and heart rate for work rates up to and including 210 W. These analyses were performed to determine whether or not these variables differed significantly among the three treatments. If significant differences were found, Duncan's multiple range test was then used to determine which of the treatment combinations were significantly different from the others.

To determine at which work rates significant differences in lactate, ventilation, and heart rate occurred, these variables were analyzed at each work rate with a 1-way ANOVA for repeated measures. If significance was found at any work rate, Duncan's multiple range test was used to ascertain which treatments were significantly different from the others.

In order to find out whether FIO_2 influenced key variables at exhaustion, differences between maximum values of oxygen uptake, lactate and ventilation were analyzed with a one-way ANOVA for repeated measures. Duncan's multiple range test was again used to determine which maximum values were different from the others if the one-way ANOVA for repeated measures indicated significance. Lactate plotted as a percentage of $\dot{\text{V}}\text{O}_2$ max for each treatment was analyzed with a two-way ANOVA for repeated measures to find out whether any lactate differences were related to percent $\dot{\text{V}}\text{O}_2$ max.

To determine if there were rate differences in lactate accumulation among the three treatments, quadratic equations were fitted to each plot of lactate accumulation. The coefficients measuring rates of change in these quadratic equations were then analyzed with ANOVA.

To determine the point at which lactate begins to rise (lactate threshold), two lines were fitted to each plot of mean lactate concentration against work rate. Different two line combinations were used until the best fit, as determined by the residual sum of squares, was found. The work rate nearest the intersection of those two lines was chosen as the breaking point.

An arbitrary decision was made to call any $p \leq .05$ significant. If $.1 > p > .05$, then this was indicated as approaching significance.

CHAPTER IV

RESULTS

Oxygen Uptake

The mean oxygen uptake values for all subjects at each work rate during the three different gas treatments are presented in the Appendix, Table 2. There was no significant difference in oxygen uptake among the three different gas treatments (see Appendix, Table 3, and Figure 1). The difference between the maximal oxygen uptake ($\dot{V}O_2$ max) of the six subjects for each of the three gas treatments approached but failed to show significance ($p = .06$) (see Appendix, Table 4). Although $\dot{V}O_2$ max was not significantly different among the three gas treatments, five of the six subjects had their highest $\dot{V}O_2$ max during the hyperoxic test. Four of the six subjects had their lowest $\dot{V}O_2$ max during hypoxia.

Blood Lactate

The mean blood lactate values of the six subjects at each work rate during the three different gas treatments are presented in the Appendix, Table 5. The differences in lactate concentrations were analyzed up to 210 W (see Appendix, Tables 6 and 7). Blood lactate concentrations were significantly different at all three gas treatment levels--with hyperoxia having the lowest lactate levels and hypoxia the highest (see Figure 2). A comparison of mean blood lactate levels at each work rate (see Appendix, Table 8) revealed that there were no

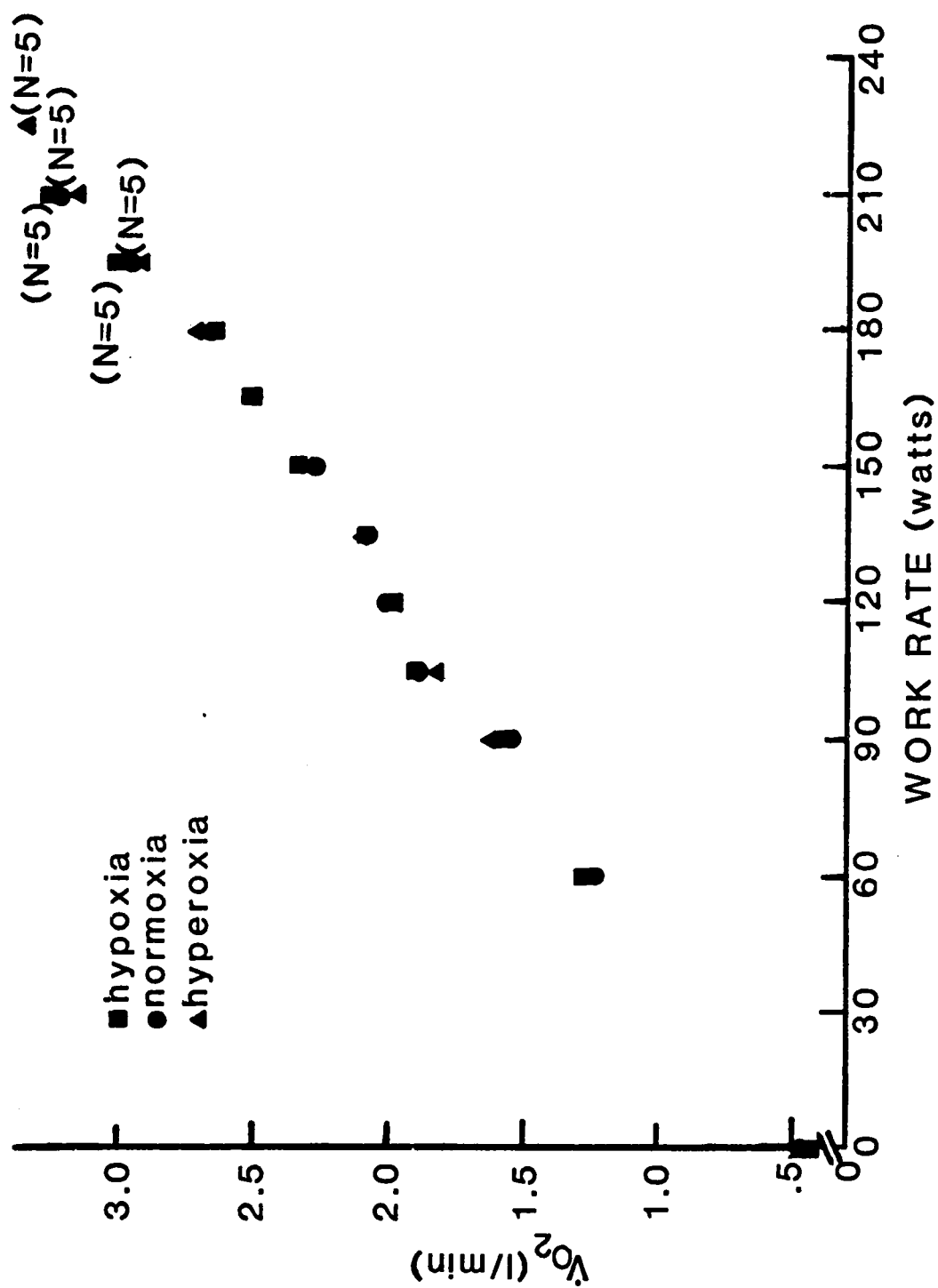


Figure 1. Mean $\dot{V}O_2$ values.

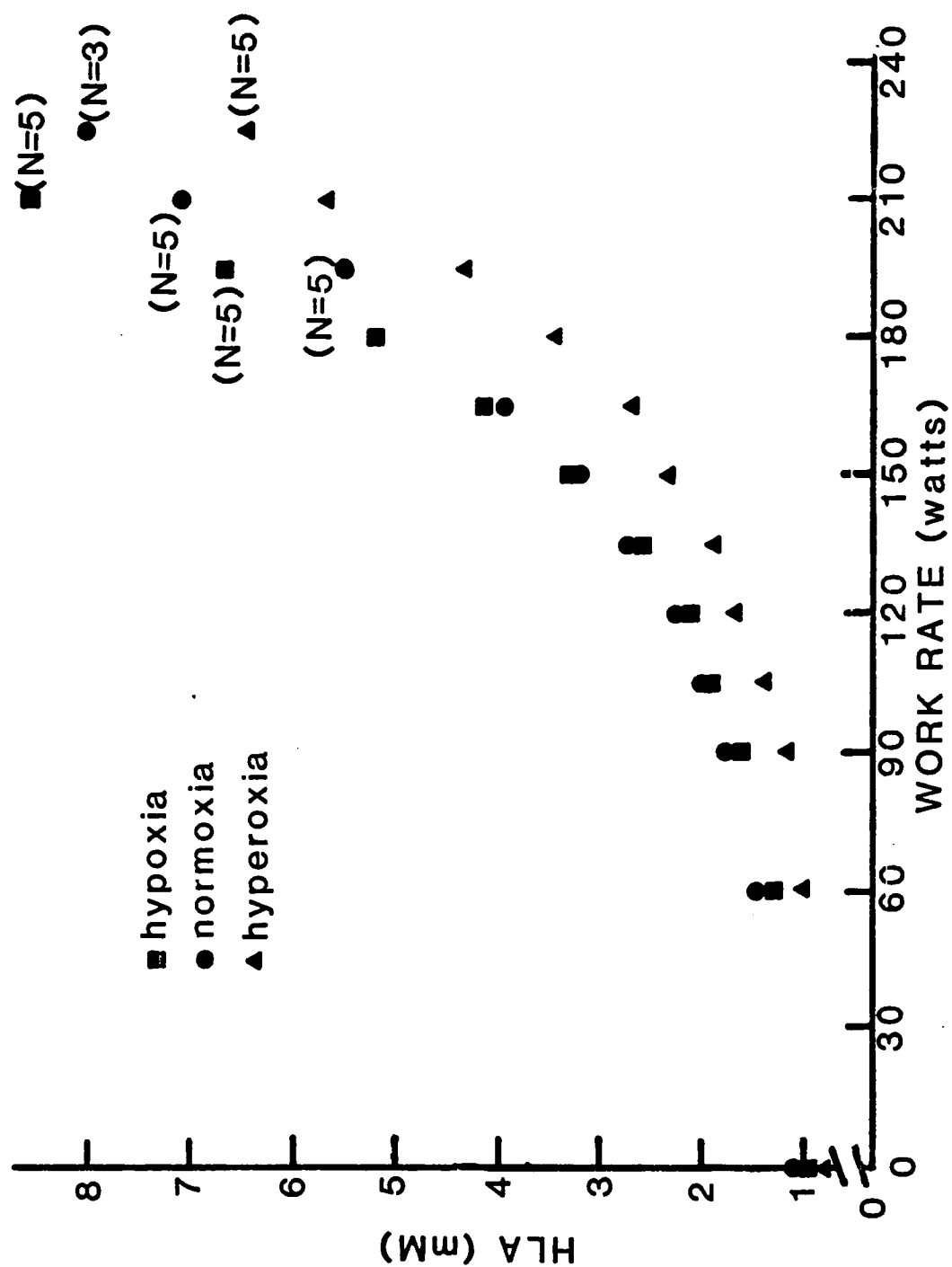


Figure 2. Mean lactate values.

significant differences in the lactate concentrations among the three gas treatments until a work rate of 120 W was reached. At this work rate and until a work rate of 200 W was reached there was significantly ($p < .0045$) lower lactate concentrations between the hyperoxic treatment and the other two treatments. At 200 W and above there were significant ($p < .004$) differences among all three gas treatments--with hyperoxia having the lowest lactate levels and hypoxia the highest. Appendix, Table 9, shows that mean maximal lactate concentrations at exhaustion for the six subjects during the three gas treatments were not significantly different ($p = .19$). Although the difference in maximal lactates was not significant, five of the six subjects had maximal lactate concentrations during hyperoxia that were less than those under normoxia or hypoxia at exhaustion. The rate at which lactate accumulated in the blood was significantly greater ($p = .03$) during the hypoxic test when compared to the hyperoxic test. This rate approached significance ($p = .07$) where hypoxia was compared to normoxia (see Appendix, Table 10). When lactate accumulation was analyzed as a function of percent $\dot{V}O_2$ max for each gas treatment, it was found that there were no significant differences in lactate concentrations among the three gas treatments (see Appendix, Table 11, and Figure 3).

When two lines were fitted to the plot of lactate concentration against work load for each gas treatment to determine the point at which lactate concentration breaks upward, a comparison of the sum of the squared residuals of the various two line possibilities showed that the "break-point" for the hypoxic and normoxic treatments

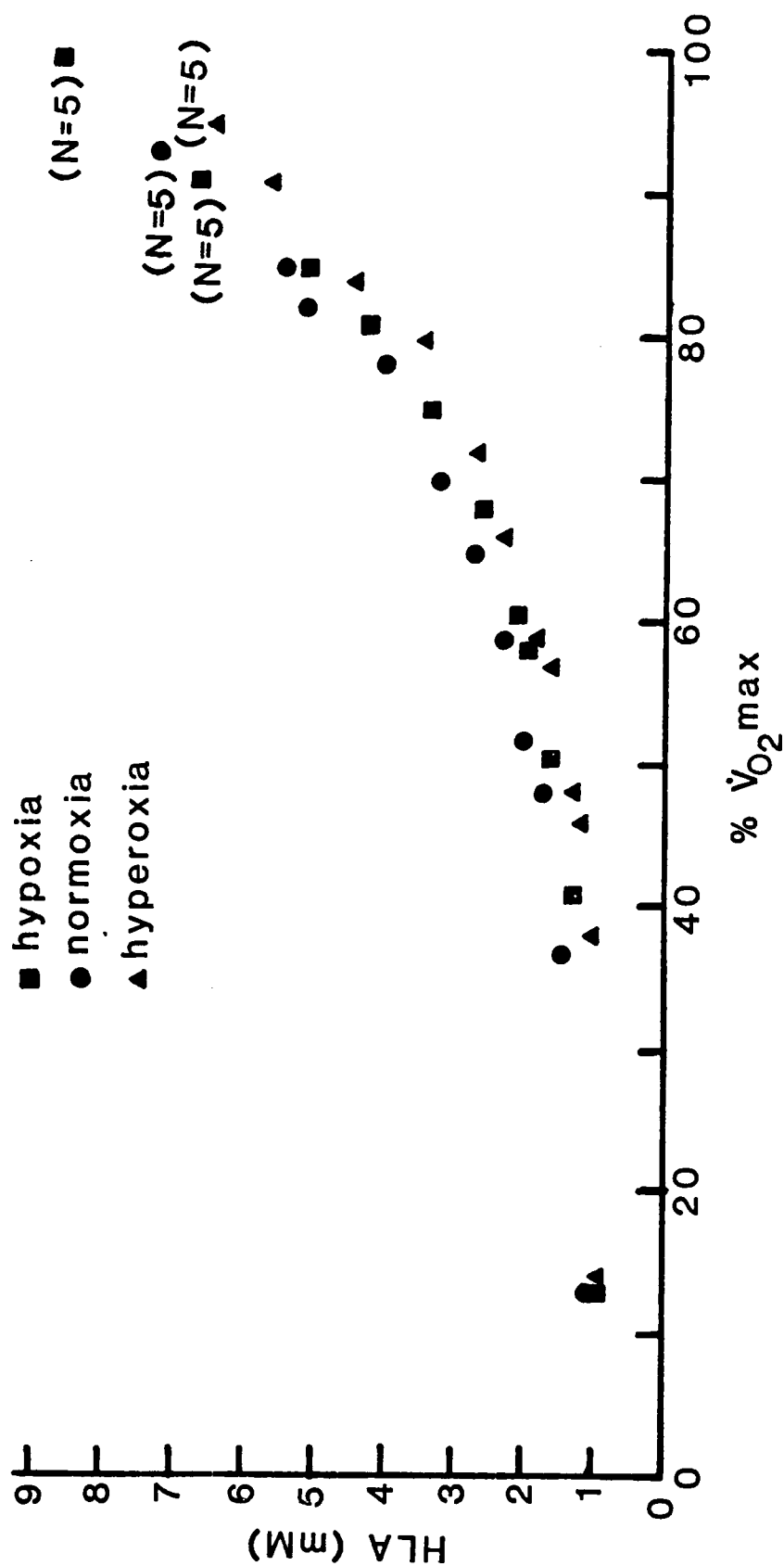


Figure 3. Plot of mean lactate values against % $\dot{V}O_2$ max.

occurred at 135 W while the "break-point" for the hyperoxic treatment occurred at 150 W.

Minute Ventilation

The mean minute ventilation values at each work rate for the six subjects during the three gas treatments are presented in the Appendix, Table 12, and Figure 4. There were significant differences at the .01 level in ventilation among all of the three gas treatments (see the Appendix, Tables 13 and 14) at work rates up to 210 W. The lowest rates of ventilation were in hyperoxia while hypoxia produced the highest. An investigation of the work rates at which minute ventilation was significantly different (see Appendix, Table 15) among the treatments reveals that at 105, 150, 195 and 210 W there are significant differences among all three gas treatments--with ventilation during hypoxia being the highest and hyperoxia being the lowest. At work rates of 135, 165 and 180 W ventilation during hyperoxia was significantly lower than either normoxia or hypoxia. The maximum ventilation at exhaustion during hyperoxia was significantly lower ($p = .001$) than either normoxia or hypoxia (see Appendix, Tables 16 and 17).

Heart Rate

Table 18 in the Appendix contains the mean heart rate values of the six subjects at each work rate during the three gas treatments. There was a significant difference when analyzed up to 210 W among all three of the gas treatments in heart rate (see Appendix, Tables 19 and 20)--with hyperoxia exhibiting the lowest and hypoxia the

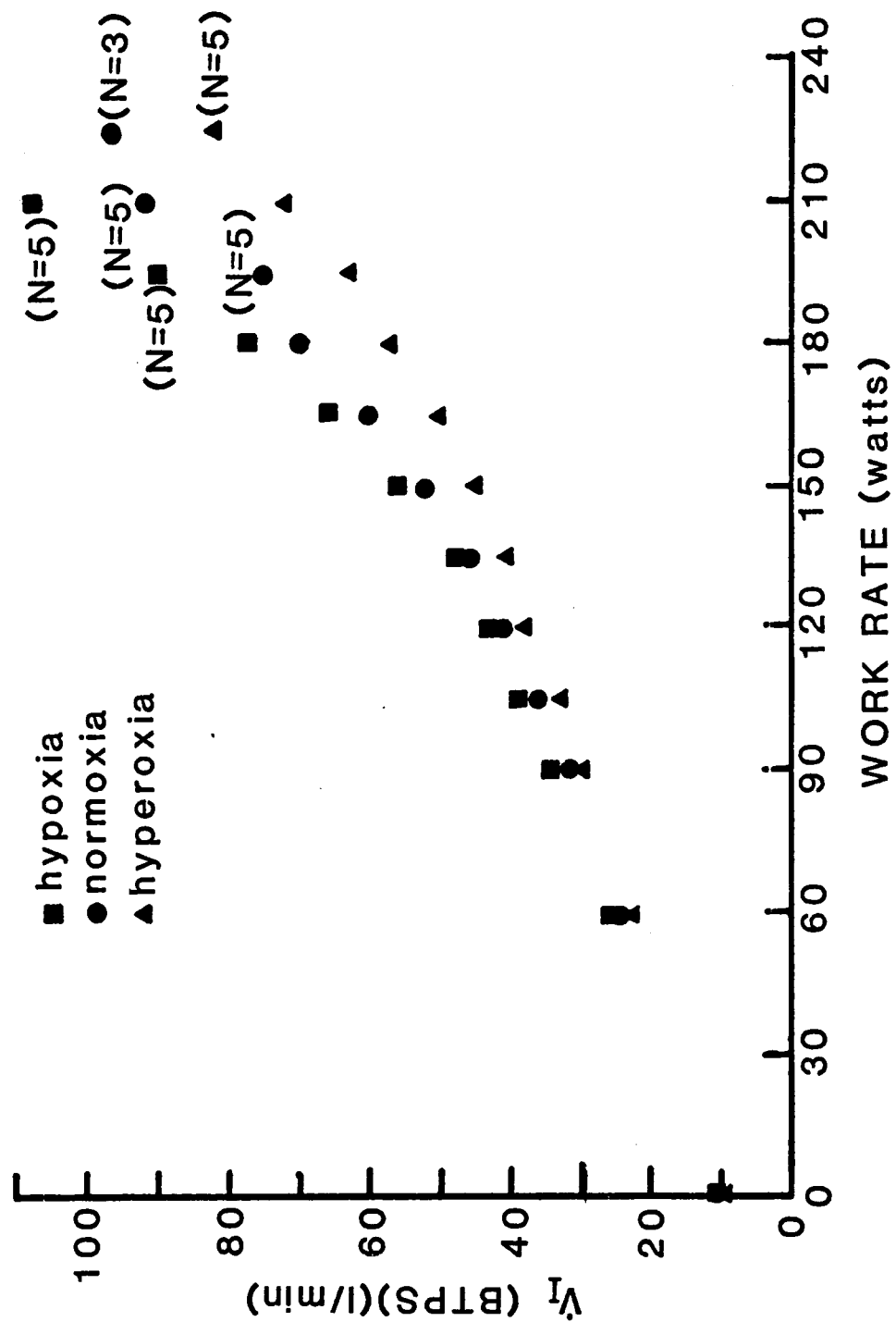


Figure 4. Mean minute ventilation values.

highest rates. This was the case even though there was not any specific work rate at which the mean heart rate during one treatment was significantly different from the others (see Appendix, Table 21).

Performance

Table 22 in the Appendix displays the work rate that each subject was able to attain during the three separate treatments. All subjects achieved their greatest work rate either during hyperoxia or normoxia. Four of the subjects reached higher work rates during hyperoxia than with either hypoxia or normoxia, while two of the subjects were able to ride to the same work rate under both normoxic and hyperoxic conditions. The lowest work rate attained was always during the hypoxic treatment.

CHAPTER V

DISCUSSION

The questions originally posed dealt with whether the "lactate threshold," the rate of lactate accumulation, and lactate concentrations at identical work rates would be altered during progressive exercise while breathing hypoxic, normoxic or hyperoxic mixtures. The answer to the lactate threshold question is not clear, because of the individual differences and ambiguity in trying to choose a "break point." The rate and concentration questions appear to be answered in a satisfactory manner. The rate of lactate accumulation during hypoxia was significantly higher than in hyperoxia, while lactate levels at identical work rates and oxygen uptakes were highest in hypoxia and lowest in hyperoxia.

The organization of this discussion will begin with some of the oxygen uptake data. This will lead directly into a discussion of the lactate results and other results.

Oxygen Uptake

Before any definitive statements could be made concerning the lactate results from these experiments, it had to be ascertained whether or not the oxygen uptake at identical work rates was similar among the three treatments. The results indicated that $\dot{V}O_2$ was in fact the same at equal work rates among the three treatments. This conflicts with those studies that reported differences in oxygen uptake at

equivalent work rates during hypoxia, normoxic and hyperoxic breathing (Jones et al., 1972; Ekblom et al., 1975), but it agrees with data that indicate no changes in oxygen uptake under these conditions (Lundin et al., 1947; Hughes et al., 1968; Adams and Welch, 1980; Stanek et al., 1979). Since the energy requirement for each 3 minute work load was the same during the three gas treatments, it would be expected that oxygen uptake among the three treatments would be similar.

Shifts in the Lactate Threshold

Almost all investigations that have examined the lactate threshold have used either non-invasive or else arbitrary determinations of this point (Davis et al., 1976; Stamford et al., 1978; Ivy et al., 1980; Wasserman et al., 1973; Pendergast et al., 1980). In this present investigation the threshold was determined by defining several possible shift points (120, 135, 150, 165, 180, and 195 W) on the plot of mean lactate accumulation against work rate. Two straight lines--one to points above and one to points below--were then fit to each shift point for each of the three treatments. The shift point for which the sum of the squared residuals of these two straight lines was smallest for each treatment was chosen as the work rate at which lactate levels begin to rise. The lactate threshold for hypoxia and normoxia occurred at a work rate of 135 W; the hyperoxic threshold occurred at 150 W. Although this method of determining a "break point" indicated differences between hyperoxia and the other two treatments, it cannot be said whether this difference was significant or not.

There are serious problems with the determination of this threshold and with the concept itself. Each subject produced very individualistic lactate curves during these tests and there was no clear cut pattern of lactate accumulation. Some subjects' blood lactate concentrations would increase quickly, while others would not increase until much later. This individual variation could be the result of differences in fiber type or recruitment, metabolic processes, or the respiratory capacity of the tissue. Therefore, it could not be established realistically for the total group data where clear breaking points occur during each treatment. It appears that the progressive rise in lactate concentration is such that a "lactate threshold" is irrelevant. It may be that the concept of "lactate threshold" is incorrect in most investigations because the rise of lactate is continual throughout the test and there is no clear "breaking point." The lactate threshold may be more valid if applied to well trained subjects that show the classic lactate response of resting levels until about 70% $\dot{V}O_2$ max. Attempts to assign absolute lactate levels to this threshold (Skinner and McLellan, 1980; Kindermann et al., 1979) are questionable, as each individual has a unique lactate response. As an example of this, during hyperoxia subject 2 was exhausted with a lactate concentration of 4.0 mM--which is an extremely low value.

Lactate Accumulation

The major finding of this investigation is that lactate concentrations during progressive exercise were significantly different

at some of the work rates during hypoxic, normoxic, and hyperoxic breathing. This indicates that at the same $\dot{V}O_2$ there were different concentrations of lactate among the three treatments. This is an important finding in that the differences in lactate concentrations seen at the same work rates in this study cannot be explained by differences in the muscle's final steady-state oxygen uptake.

Analyses of the lactate data collected in this study revealed that the rate of lactate accumulation was significantly different among the three treatments. The rate at which lactate accumulated in the blood was significantly higher ($p = .03$) during hypoxia when compared to hyperoxia, and it approached significance ($p = .07$) when hypoxia was compared to normoxia. This indicates that differences seen in the concentration of lactate at equal work rates during the three tests were not simply caused by differences in the resting levels of lactate. Although the rate at which lactate levels rose was not significantly different when comparing normoxia to hyperoxia, the hyperoxic rate was less.

It now becomes necessary to examine the possible explanations of these lactate data. Since the oxygen uptakes at equal work rates were equivalent it is very difficult to explain the cause of the lactate differences seen at identical work rates. Any increase in lactate seen in the blood should be a result of either excess production of pyruvate as suggested by Huckabee (1958), or hypoxia of the working tissue. The question then becomes one of whether the variation in $FI O_2$ altered the availability of oxygen at the cellular

level or whether there was some change in the amount of excess pyruvate at identical work rates.

In accordance with data presented in the literature review, there was little rise in lactate values above resting concentrations until approximately 60% of $\dot{V}O_2$ max at all values of FI_{O_2} . At 120 W, hyperoxic lactate levels were significantly less than for either of the other two treatments, and it was not until 195 W that the lactate levels were significantly different among all three gas fractions. It is obvious from these data that whether the effect of altering FI_{O_2} was on the availability of oxygen at the cellular level or on the amount of excess pyruvate produced, the result was only apparent at the moderate to intense work rates. This is consistent with data that reveal no increases above resting levels in muscle lactate concentration at low work rates (Karlsson, 1971).

At the moderate-to-intense work rates, where this study revealed differences in lactate concentration, it might be argued that the lactate differences seen are not necessarily due to alterations in production rates from either varied rates of pyruvate overflow or tissue hypoxia--but rather that the rate of release, distribution or removal of lactate is changed by the different treatments of FI_{O_2} . Although there is inadequate research in this area, the results of other investigations show little change in these parameters when the FI_{O_2} is altered (Linarsson et al., 1974; Lewis, 1973). In a study investigating the effect of altering the PO_2 on muscle lactate, Linarsson et al. (1974) found that lactate levels within the working

muscle itself are different at submaximal work rates depending on the PO_2 . This would be evidence that it is the production of lactate that leads to the varied concentrations found. However, Linarsson et al. used a hyperbaric chamber to produce conditions of hypoxia and hyperoxia. The effect of altering the atmospheric pressure during exercise on lactate dynamics is not known. Their results must therefore be interpreted with caution. However, the blood lactate data of Linarsson and his group are similar to other blood lactate results collected during investigations using gas mixtures to produce hypoxia and hyperoxia. Therefore the muscle lactate values measured in their study may be of some value in explaining results from this investigation.

If indeed the different lactate levels measured at equal oxygen uptakes during this study were due to dissimilar rates of lactate production, it is necessary to examine what factors might be affected by alterations in the FI_{O_2} that would lead to these differences in lactate production. Since the working tissue was able to achieve the same rate of oxygen uptake at each work rate in each of the three tests, it is possible that the differences in lactate levels might be explained by alterations in the time it took for the muscle to reach a steady state of oxygen uptake.

Data from this present investigation show that with each 15 W increase in work rate, the oxygen demand of the working tissues was raised 200 ml/min. This was accompanied with average increases in ventilation of 8 liters/min and heart rate of 7 beats/min. Hagberg et al. (1978) has found that the $\dot{V}O_2$ half-time (half of the time

required for attaining a steady state of oxygen uptake) is approximately 11 seconds from the initiation of exercise to a work rate of 50 W. From these measurements it can be estimated, although there are no data verifying this, that an increment of 15 W that occurs during the midst of exercise while all of the oxidative machinery is turned on should have a half-time of less than 11 seconds. The time required for the oxygen uptake to reach a new steady state in this test would then be in the order of 20-30 seconds. At higher intensities the time to steady state will be somewhat longer because of a slower rate of adjustment of heart rate and ventilation to the final value with increasing work rates (Broman and Wigertz, 1971).

If there was a 20-30 second lag time for a steady-state of oxygen uptake to be achieved, then the amount of lactate that theoretically should have been produced during this time period can be calculated. This is accurate if the majority of the ATP used during this time period was resynthesized through the process of lactate formation. As an example of this, the shift in work rate from 150 to 165 W will be examined. This work rate increase occurred just at the "lactate threshold," so that the time to achieve an oxygen uptake steady-state will be slightly higher (Whipp and Wasserman, 1972). A 30 second oxygen deficit would be considered maximal, so this figure will be used. Since the oxygen uptake increase was 200 ml/min, a 30 second lag produced a 100 ml, or about 4 mmol, oxygen deficiency. A 4 mmol oxygen deficiency is equivalent to 24 mmol of ATP. This amount of ATP must have been formed anaerobically through the production of 8 mmol of lactate from glycogen. The lactate was then distributed

through the approximately 44 l of body water in each subject for a 0.17 mM increase in the blood lactate. But the actual rise in blood lactate from 150 to 165 W averaged 0.7 mM among the treatments.

All of the other lactate increases measured as the work rate changed were also greater than that which should have occurred. This is good evidence that the lactate increases in this investigation cannot be completely explained by transient tissue hypoxia. Therefore the differences in lactate concentrations seen at identical work rates among the three gas treatments were not simply a result of differences in the time required to achieve a steady-state of oxygen uptake, as suggested by some investigators (Linnarsson et al., 1974; Gautier et al., 1978; Hughes et al., 1968). The hypotheses that there may have been differences in excess pyruvate formation or that there may have been differences in the rate of production and removal of lactate within the muscle are alternate explanations of these lactate results.

The Pasteur effect may be important in this study. Rapoport et al. (1976) showed that increasing the oxygenation of red blood cells decreases their rate of glycolysis. A similar effect, but in the opposite direction, was shown by lowering the P_{O_2} of red blood cell suspensions (Hamasaki et al., 1969). If the higher P_{O_2} does inhibit glycolysis, this could explain the lower values seen during the hyperoxic tests. Rapoport and his group suggested that the enzyme phosphofructokinase is the glycolytic regulator which is stimulated or inhibited by changes in P_{O_2} . Although there are no reports in the literature of this effect on human skeletal muscle, it is a likely hypothesis that P_{O_2} does have some influence on the rate of glycolysis.

While this study provides no direct evidence bearing on the likelihood of this hypothesis, it cannot be discounted as an explanation of the varied lactate concentrations at similar oxygen uptakes.

A feasible explanation of these lactate data involves the type of fuel being used by the working tissue. The use of free fatty acids as the fuel source lessens the possibility of lactate production. At the low intensities of work, free fatty acids predominate as the major fuel source (Newsholme, 1977)--while as the intensity of work increases, carbohydrates are utilized to a greater extent. As carbohydrate metabolism is increased, there is a greater possibility for lactate production--presumably through an elevated pyruvate overflow. If varying the FIO_2 could alter the ratio at which these fuels are metabolized--with hyperoxia increasing the utilization of fats and hypoxia decreasing this utilization--then this might explain the elevated lactate levels seen in hypoxia and the decreased levels that were measured during hyperoxic breathing. The R values that were measured during these experiments would support this hypothesis. R values recorded were much lower during hyperoxic breathing when compared to the other two treatments. This might indicate a heightened free fatty acid metabolism and explain the lower lactate values. Although the possibility exists that the lower R measured during hyperoxic exercise is due to decreased CO_2 elimination (Gautier et al., 1978), Wilson and Welch (1975) suggested that this is not the case. There are few studies that have investigated this hypothesis of altered fuel utilization, but Jones et al. (1972) found an increased utilization of free fatty acids during hypoxic breathing--which is

contrary to the ideas just presented. Linnarsson et al. (1974) showed that glycogen depletion in working muscle is similar during hyperbaric hypoxia, normoxia and hyperoxia. This suggests that fuel utilization is similar with altered values of $\dot{V}O_2$. More research is necessary to resolve this issue.

The possibility exists that hormonal regulation of metabolism could be shifted by alterations in $\dot{V}O_2$. Hesse et al. (1981) found a decrease in catecholamines during exercise when subjects breathed 100% oxygen. It is uncertain whether the 25% decrease in catecholamines seen in their study could explain the major alterations in muscle metabolism as seen in this study.

$\dot{V}O_2$ max

An explanation of the $\dot{V}O_2$ max data from this investigation is necessary. Although $\dot{V}O_2$ max was not significantly different among the three gas conditions, the probability bordered on significance ($p = .06$). The means of 3.14 l/min during hypoxia, 3.27 l/min during normoxia, and 3.45 l/min during hyperoxia show a clear trend toward a higher $\dot{V}O_2$ max when breathing a hyperoxic gas mixture. Quite a few investigators have reported higher $\dot{V}O_2$ max during hyperoxic breathing (Ekblom et al., 1975; Linnarsson et al., 1974; Welch et al., 1974; Hughes et al., 1968). Most of these studies have utilized Douglas bag techniques which may record falsely high oxygen uptakes during hyperoxia (Welch and Pederson, 1981). Several studies have also shown lower $\dot{V}O_2$ max values during hypoxic breathing (Ekblom et al., 1975; Fagraeus et al., 1973), but most studies utilizing mild hypoxic

breathing, with an FI_{O_2} above 16%, have shown that $\dot{V}O_2$ max is not altered (Bason et al., 1971; Adams and Welch, 1980).

That the $\dot{V}O_2$ max results of this investigation approach significance can perhaps be explained by the maximal work rates attained by each subject during the three different gas conditions. At near maximal work rates, the mean oxygen uptake values during the three treatments were equivalent. A subject's $\dot{V}O_2$ max varied only if the maximal work rate attained was different among treatments. Four of the six subjects were able to ride for at least one more work rate during hyperoxia than normoxia or hypoxia. The other two subjects were able to ride for one work rate more during both hyperoxia and normoxia when compared to hypoxia. So all subjects were able to ride to greater work loads while breathing hyperoxic or normoxic mixtures when compared to the hypoxic mixture.

For a subject to ride for an extra 3 minutes at an increased work load requires a greater amount of oxygen. None of the subjects exhibited the classical plateau effect in maximal oxygen uptake that occurs when a subject continues to exercise at a maximal rate without a concomitant oxygen uptake increase. For extra work to be done in this study, an increase in oxygen uptake was necessary. Since four of the six subjects attained their highest work rates during hyperoxia, it would follow that their $\dot{V}O_2$ max should be higher during hyperoxia. The question of whether the subjects in this study were able to ride longer because of the slight increase in $\dot{V}O_2$ max or whether the subjects' $\dot{V}O_2$ max increased because work could be maintained for a longer time cannot be answered from this research.

It should be noted here that the slight increase in $\dot{V}O_2$ max during hyperoxia and the decrease during hypoxia cannot be due to adjustment of ventilatory or cardiac parameters. Mean ventilation was significantly less at exhaustion during hyperoxia, even though $\dot{V}O_2$ max was slightly higher, when compared to either normoxia or hypoxia. The maximal heart rates were almost identical.

A possible explanation of these $\dot{V}O_2$ max data is that during hyperoxia there is either an increased oxygen delivery to the working tissue or an increased oxygen extraction by the working tissue. But research dealing with these possibilities is scarce. Welch et al. (1977) found no difference in oxygen transported to the exercising muscle at $\dot{V}O_2$ max. Hughes et al. (1968) obtained similar results as Welch et al., except that they also estimated oxygen delivery in hypoxia at exhaustion and found it to be similar to conditions of normoxia and hyperoxia. Ekblom et al. (1975), on the other hand, calculated that oxygen delivery was decreased during hypoxia and increased during hyperoxia at exhaustion. The issue is further clouded by data from Doll et al. (1968) which suggest that even at $\dot{V}O_2$ max oxygen delivery during normoxia is more than adequate. This question is far from settled, as is the question of whether or not oxygen extraction may be increased during hyperoxia.

Lactate vs. Percent $\dot{V}O_2$ max

Another observation to be made concerning the lactate results is that even though the mean values for $\dot{V}O_2$ max were not significantly different among the three treatments, the plot of lactate vs. percent

$\dot{V}O_2$ max indicates no significant difference in lactate concentrations among the three treatments. This suggests that lactate concentrations are somehow related to the percent $\dot{V}O_2$ max at any given time. Therefore lactate concentrations were approximately the same at 80% $\dot{V}O_2$ max--no matter what the treatment. This is somewhat in conflict with the fact that the lactate concentrations at identical oxygen uptakes were quite different. But since differences in $\dot{V}O_2$ max among the three treatments approached significance, this shift is not unexpected. This is similar to studies that have shown that at altitude (hypoxia) lactate values are increased at the same absolute work rates compared to sea level (normoxic conditions), but when lactate is plotted as a function of percent $\dot{V}O_2$ max there are no differences (Hermansen and Saltin, 1967). Although there is the problem of resolving how the relative rate at which the muscle is working determines lactate output, it is still important to note that lactate levels parallel the relative rate at which the muscle is working. This would give some support to the hypothesis that work of this type is limited by acid-base changes--especially since lactate concentrations at exhaustion during this investigation were not significantly different among treatments.

Performance and Acid-base Balance

Excessive accumulation of lactate during exercise is postulated to be a factor leading to the development of fatigue (Karlsson et al., 1975; Tesch, 1980; Karlsson, 1971; Asmussen et al., 1948). Karlsson et al. (1975) showed that increased lactate levels induced by previous

exercise of certain muscles negatively affected performance capacity of other muscles. Linnarsson et al. (1974), using a hyperbaric chamber, added support to this hypothesis when they found the same lactate concentrations within the working muscle at exhaustion during hypoxic, normoxic and hyperoxic breathing, even though the time to exhaustion in each case was different. But other investigators measuring blood lactate have found different lactate levels at exhaustion at various inspired oxygen fractions (Hughes et al., 1968; Adams and Welch, 1980; Lundin and Strom, 1947).

Lactate concentration and muscle pH have a close relationship at the termination of exercise (Sahlin, 1978). Other research has revealed that exercise performed during induced metabolic acidosis decreases performance (Jones et al., 1977). Fitts and Holloszy (1976) demonstrated that a linear relationship exists between lactate concentration and twitch and tetanic tension in stimulated frog satorius muscle. The mechanism by which lactate accumulation within the working muscle might lead to impaired muscular function and exhaustion is through an increase in the hydrogen ion concentration. The decreased pH has been shown to lead to a lowered activity of phosphofructokinase (McGilveray, 1973). High hydrogen ion concentrations have also been shown to decrease the number of calcium ions bound to troponin during the excitation coupling and thereby decrease contractile force through a reduction in the number of active actomyosin interactions (Nakamura and Schwartz, 1972).

It is likely that during exercise in which there is a lactate accumulation there is a relationship between muscle lactate formation

and fatigue. Research has shown that while breathing hypoxic, normoxic or hyperoxic gas mixtures, exhaustion during maximal exercise occurred at the same plasma pH but at different blood lactate levels (Adams et al., 1980; Kaijser, 1970). Adams and Welch (1980) postulated that alterations in FI_{O_2} lead to different rates of lactate production. The varied hydrogen ion concentrations produced are then buffered by different rates of respiratory compensation. In hypoxia, lactate is produced at an increased rate. This is compensated by a higher rate of ventilation. During hyperoxic breathing the opposite occurs. The end result is that during hypoxia, even with the increased ventilation, the critical muscle pH is reached sooner than in normoxia or hyperoxia. During hyperoxia the critical pH level is not reached until a later time. This could explain the differences seen in performance in mild hypoxia, normoxia and hyperoxia even when maximal oxygen uptakes are similar (Hughes et al., 1968; Adams and Welch, 1980; Wilson and Welch, 1975; Ekblom et al., 1975).

The data from this present investigation are in accordance with these results. At exhaustion, blood lactate concentrations were not significantly different from one treatment to another. In the case of Subject 4, exhaustion during hypoxia occurred at 225 W with a lactate concentration of 8.9 mM. Exhaustion during normoxic breathing occurred 6 minutes later at 255 W and 7.7 mM. Exhaustion during the hyperoxic treatment occurred at 270 W and 7.6 mM. In this subject exhaustion during hyperoxia occurred 9 minutes later at a work rate 45 W above and with a lactate concentration less than exhaustion during hypoxia. It is reasonable that this extra work was accomplished as a result of

the lessened accumulation of lactate during hyperoxia. It may be that the increases in performance levels seen in this study are due to the acid-base changes described previously. The possibility does still exist, though, that in this subject changes in performance were due to the subject's ability to achieve a higher $\dot{V}O_2$ max during hyperoxia. Unfortunately, the design of this investigation did not permit a more careful analysis of these discrepancies.

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APPENDIX

APPENDIX

Table 1. Subject personal data.

Subject	Test* Order	Age (Yrs)	Weight (Kg)	Max $\dot{V}O_2$	
				L/min	ML/KG·Min
1	1,2,3	23	71.0	3.32	46.7
2	2,1,3	20	72.5	3.18	43.8
3	3,1,2	44	68.0	3.15	46.3
4	2,3,1	28	84.1	3.75	44.6
5	1,3,2	20	69.2	3.34	48.3
6	3,2,1	31	73.5	3.77	51.3
$\bar{X}$		27	73.1	3.42	46.8

*1 = hypoxia, 2 = normoxia, 3 = hyperoxia.

Table 2. Mean oxygen uptake values for all subjects at each work rate (WR). Values expressed as liters O₂ per minute. N=6 unless otherwise noted.

WR (Watts)	0	60	90	105	120	135	150	165	180	195	210	225	240	255	270
Gas treatment															
17%	.40	1.30	1.58	1.80	1.91	2.10	2.33	2.51	2.64	3.01 n=5	3.27 n=5	3.25 n=2			
21%	.43	1.21	1.54	1.73	1.91	2.08	2.26	2.53	2.65	2.90 n=5	3.20 n=5	3.32 n=3	3.56 n=2	3.63 n=1	
60%	.49	1.27	1.60	1.67	1.97	2.07	2.31	2.53	2.76	2.90	3.13	3.32 n=5	3.45 n=2	3.56 n=1	3.87 n=1

Table 3. Covariance analysis of oxygen uptake.

Source	DF	Sum of Squares	F Value	Prob F
FI _O ₂	2	.0298	.99	.3742
WR	1	138.9125	9.98.93	.0001
Error	207	3.1258		
Total	210	142.0882		

Table 4. Repeated Measures ANOVA for Mean $\dot{V}O_2$ max.

Source	DF	Sum of Squares	F	Prob F
Subjects	5	1.3997		
Treatment (FI _O ₂)	2	.3024		
Treatment Error	10	.4067	3.72	.062
Total	17	2.1089		

Table 5. Mean blood lactate values for all subjects at each work rate (WR). Values expressed as mmole/liter. N=6 unless otherwise noted.

WR (Watts)	0	60	90	105	120	135	150	165	180	195	210	225	240	255	270
Gas treatment															
17%	.99	1.28	1.61	1.93	2.11	2.58	3.30	4.15	5.12	6.64 n=5	8.58 n=5	7.72 n=2			
21%	1.16	1.44	1.71	1.97	2.29	2.73	3.23	3.96	5.10	5.46 n=5	7.16 n=5	8.01 n=3	6.42 n=2	7.68 n=1	
60%	.91	1.00	1.19	1.34	1.64	1.87	2.33	2.66	3.41	4.32	5.67	6.46	4.46 n=2	6.00 n=1	7.55 n=1

Table 6. Repeated measures ANOVA for lactate concentrations at three gas levels and work rates $\leq$ 210 watts.

Source	DF	Sum of Squares	F
Subject	5	129.0072	
Treatment (FI0 ₂)	2	47.9731	
Treatment Error	10	7.0266	34.136**
Work Rate	10	579.2573	
Work Rate Error	50	105.5385	27.442**
Treatment by WR	20	28.5143	
Treatment by WR Error	96		10.44**
Total	193	936.8471	

* $P < .05$.

** $P < .01$.

Table 7. Results of Duncan's Multiple Range tests for lactate concentration. Means with the same letter are not significantly different. Significance level = .05.

Treatment (FI0 ₂)	Mean (mM)	N	Grouping
17%	3.35	64	A
21%	3.16	64	B
60%	2.37	66	C

Table 8. Results of repeated measures ANOVA for lactate concentration at each work rate ≤ 210 watts. P must be less than .05/11 = .0045 to indicate significance.

WR (Watts)	0	60	90	105	120	135	150	165	180	195	210
P	.4606	.0687	.0695	.0176	.002	.001	.0006	.0001	.0002	.0006	.0009

Table 9. Results of Duncan's Multiple Range tests for lactate concentration at each work rate. Means with the same letter are not significantly different. Significance level = .05.

Gas Treatment	WR (Watts)										
	0	60	90	105	120	135	150	165	180	195	210
17%	A	A	A	A	A	A	A	A	A	A	A
21%	A	A	A	A	A	A	A	A	A	B	B
60%	A	A	A	A	B	B	B	B	B	C	C

Table 10. Repeated measures ANOVA for mean maximal lactate concentration at exhaustion.

Source	DF	Sum of Squares	F	P
Subject	2	61.6693		
Treatment (FI0 ₂)	5	6.3996	1.94	.1937
Treatment Error	10	16.4671		
Total	17	84.5360		

Table 11. Summary table to determine rate differences among treatments for lactate accumulation.

Source	DF	Sum of Squares	F	P
17% vs. 60%	1	8.4751	4.59	.033
17% vs. 21%	1	6.0134	3.26	.072
21% vs. 60%	1	.1679	.09	.763

Table 12. Repeated measures ANOVA for lactate concentration as a function of percent $\dot{V}O_2$ max.

Source	DF	Sum of Squares	F
Subject	5	108.9843	
Treatment (FI_{O_2})	2	9.0440	
Treatment Error	10	29.9091	1.51
% $\dot{V}O_2$ max	16	775.0341	
% $\dot{V}O_2$ max Error	64	86.0658	36.54**
Treatment by % $\dot{V}O_2$ max	28	6.0756	
Treatment by % $\dot{V}O_2$ max Error	82	32.9842	.54
Total	207	1212.1618	

* $P < .05$.

** $P < .01$.

Table 13. Mean values for minute ventilation at each work rate. Values expressed as liters per minute. N=6 unless otherwise noted.

WR (Watts)	0	60	90	105	120	135	150	165	180	195	210	225	240	255	270
Gas treatment															
17%	11.0	26.1	34.3	39.6	43.1	48.3	56.4	66.4	77.0	90.1 n=5	108.8 n=5	105.5 n=2			
21%	9.9	24.4	31.8	36.4	41.0	46.3	52.3	60.6	69.9	74.7 n=5	92.0 n=5	96.8 n=3	103.3 n=2	109.6 n=1	
60%	10.2	22.9	30.6	33.4	38.4	40.5	45.4	49.6	57.0	63.4	72.6	82.4 n=5	74.3 n=2	83.1 n=1	91.8 n=1

Table 14. Repeated measures ANOVA for minute ventilation. Work rate $\leq$ 210 watts.

Source	DF	Sum of Squares	F
Subject	5	1239.4385	
Treatment (FI _O ₂)	2	5103.8015	
Treatment Error	10	452.6008	56.38**
Work Rate	10	86118.4066	
Work Rate Error	50	2043.4907	210.71**
Treatment by Work Rate	20	3642.1921	
Treatment by Work Rate Error	96	685.8954	25.49**
Total	193	105800.1336	

* $P < .05$.

** $P < .01$.

Table 15. Results of Duncan's Multiple Range tests for minute ventilation at work rates $\leq$ 210 watts. Means with the same letter are not significantly different. Significance level = .05.

Treatment	Mean	Grouping
17%	53.28	A
21%	47.96	B
60%	42.20	C

Table 16. Results of repeated measures ANOVA for minute ventilation at each work rate.
P must be less than .0045 to indicate significance.

WR (Watts)	0	60	90	105	120	135	150	165	180	195	210
P	.488	.068	.008	.001	.009	.003	.001	.001	.001	.001	.001

Table 17. Results of Duncan's Multiple Range tests for minute ventilation at each work rate.
Means with the same letter are not significantly different. Significance level = .05.

Gas Treatment	0	60	90	105	120	135	150	165	180	195	210
17%	A	A	A	A	A	A	A	A	A	A	A
21%	A	A	A	B	A	A	B	A	A	B	B
60%	A	A	A	C	A	B	C	B	B	C	C

Table 18. Repeated measures ANOVA for mean maximal minute ventilation at exhaustion.

Source	DF	Sum of Squares	F	P
Subjects	5	933.1889		
Treatment (FI _O ₂)	2	1828.4812	17.38	.0006
Treatment Error	10	525.9841		
Total	17	3287.6543		

Table 19. Results of Duncan's Multiple Range tests for mean maximal minute ventilation at exhaustion. Means with the same letter are not significantly different. Significance level = .05.

Treatment	Mean	Grouping
17%	108.2600	A
21%	105.1800	A
60%	85.5066	B

Table 20. Mean heart rate values for all subjects at each work rate. Values expressed as beats per minute. N=6 unless otherwise noted.

WR (Watts)	0	60	90	105	120	135	150	165	180	195	210	225	240	255	270
Gas treatment															
17%	79	107	117	127	136	144	153	163	170	175 n=5	182 n=5	183 n=2			
21%	78	101	116	124	134	142	150	159	166	169 n=5	177 n=5	179 n=3	185 n=2	186 n=1	
60%	73	101	114	122	130	137	146	153	161	167	173	177 n=5	180 n=2	180 n=1	182 n=1

Table 21. Repeated measures ANOVA for heart rate. Work rate $\leq$ 210 watts.

Source	DF	Sum of Squares	F
Subject	5	6762.2789	
Treatment (FI02)	2	1569.2283	
Treatment Error	10	1825.9811	4.29*
Work Rate	10	161909.9856	
Work Rate Error	50	3734.8552	216.76**
Treatment by Work Rate	20	467.5838	
Treatment by Work Rate Error	96	1366.0189	1.64*
Total	193	184746.2232	

* $P < .05$.

** $P < .01$.

Table 22. Results of Duncan's Multiple Range tests for heart rate at work rates ≤ 210 watts.
Means with the same letter are not significantly different. Significance level = .05.

Treatment	Mean	Grouping
17%	140	A
21%	136	B
60%	134	C

Table 23. Results of repeated measures ANOVA for heart rate at all work rates ≤ 210 watts.
P must be less than .0045 to be significant.

WR (Watts)	0	60	90	105	120	135	150	165	180	195	210
P	.499	.169	.416	.159	.199	.134	.108	.045	.016	.021	.009

Table 24. Maximal work rates attained by each subject for each treatment. Values expressed as watts.

Subject	1	2	3	4	5	6
Gas treatment						
17%	210	225	180	225	210	210
21%	225	240	180	255	210	210
60%	225	240	210	270	225	225

VITA

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