IS HYPOXIC (ALTITUDE) TRAINING MORE EFFECTIVE THAN SEA LEVEL TRAINING FOR COMPETITION AT SEA LEVEL?

BY

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ABSTRACT

The purpose of the present study was to determine whether or not the combination of normobaric hypoxia and exercise training would enhance adaptations in skeletal muscle over and above that which occurs with the same amount of training under normoxic conditions. Also investigated was the effect of such training conditions on performance as assessed by $\dot{V}O_2max$ and maximal aerobic capacity (MAC).

Ten males performed unilateral cycle ergometry training 3 times per week for 8 weeks so that one leg was trained under normoxic conditions and the other while breathing an hypoxic gas mixture (FIO_2= 13.5%; equivalent to an altitude of 3,292 meters). Absolute power output was kept constant for both conditions and subjects performed both continuous (75% pre-training maximal power output) and interval (100% pre-training maximal power output) training. Needle biopsies were taken from the vastus lateralis of both legs to assess pre- and post-training differences in morphometric and biochemical data. Performance measures included $\dot{V}O_2max$ and MAC (time to fatigue at 95% pre-training maximal power output) for each leg.

Significant increases in $\dot{V}O_2max$ ($p < 0.05$) occurred in both legs with higher peak ventilation and blood lactate

concentrations ($p < 0.05$) post-training. Marked improvements ($p < 0.05$) in MAC were also seen with an increase of 402% in the normoxically-trained leg and 513% in the hypoxically-trained leg. Citrate synthase (CS), succinate dehydrogenase, and phosphofructokinase activity was significantly ($p < 0.05$) higher in both legs following training with a significantly greater ($p < 0.05$) increase in CS in the hypoxically-trained leg. There were no differences in capillary/fiber ratio, capillary density, fiber area, fiber type, and mitochondrial volume density for either condition, pre- or post-training. It is concluded that hypoxia enhanced the muscle oxidative capacity (as marked by CS activity) but was unable to improve performance over and above that which occurs with the same training at sea level.

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CHAPTER I

1.1 INTRODUCTION

The progressive decline in maximal oxygen consumption ($\dot{V}O_2max$) and work capacity which occurs with ascent to increasing levels of altitude is well documented (Buskirk et al, 1967; Cerretelli, 1976; Pugh et al, 1964; West et al, 1983a). It is also known that acclimatization to a given altitude moderates decrements in exercise performance (Maher et al, 1974). Acclimatization has been shown to increase work capacity and produce lower levels of blood lactate, at the same power output, compared to the acute altitude exposure. There is however, little or no increase in $\dot{V}O_2max$ with prolonged exposure to altitude (Maher et al, 1974). These results suggest that adaptations have occurred at the muscle level, independent of changes in oxygen delivery. Moreover, whether or not such adaptations might result in enhanced performance upon return to sea level is not known. In spite of this, widespread support for this theory exists among coaches and athletes who sojourn to altitude in preparation for sea level competition.

This review will focus on the reduced partial pressures of oxygen at different levels of altitude, its

acute effects, adaptations through chronic exposure to altitude, and the influences on performance at both altitude and sea level.

1.2 PARTIAL PRESSURE OF OXYGEN AT ALTITUDE

Hypoxia can be defined as occurring when oxygen is delivered to the tissues at a partial pressure lower than it would be at sea level. Because the partial pressure of oxygen in ambient air decreases proportionally with the decreasing barometric pressure, lower partial pressures are found above sea level. The fractional concentration of oxygen remains the same (20.93%), but the number of oxygen molecules per unit volume decreases with ascent to altitude. Therefore, a greater pulmonary ventilation is necessary in order to deliver the same number of oxygen molecules as at sea level. The fundamental physiological effects of ascending to altitude are thus caused by a reduction in the partial pressure of oxygen (PO_2). The oxygen tension of the alveolar air (PAO_2), and subsequently the oxygen tension of arterial blood (PaO_2), is determined by the magnitude of the pulmonary ventilation in addition to the composition and pressure of the inspired air (Åstrand et al, 1986) (Table 1).

TABLE 1: The effect of altitude on PO_2

Altitude (m. above Sea Level)	Barometric Pressure (mmHg)	PO_2(Air) (mmHg)	PO_2(Alveoli) (mmHg)	PO_2(Arterial) (mmHg)
0	760	159	105	100
1,000	674	131	79	83
2,000	596	124	84	80
3,000	526	109	74	70
4,000	462	97	62	58
5,000	405	85	51	47
6,000	354	75	42	37
7,000	308	66	32	28
8,000	267	58	27	24

(Modified from Åstrand et al, 1986)

The arterial PO_2 is constantly lower than the alveolar PO_2 because of the diffusion limitation of oxygen transfer across the blood barrier of the lung (West et al, 1983b). As the PaO_2 is reduced, the effective slope of the oxyhemoglobin dissociation curve is increased and for any given drop in PO_2, the associated decrease in oxygen saturation (SaO_2) will be much larger (Bebout et al, 1989). This inability to fully, or even optimally, saturate oxygen decreases the blood's oxygen carrying capacity.

When oxygen deprivation reaches a critical level the ventilatory chemoreceptors become activated resulting in an increase in ventilation ($\dot{V}e$) which partially defends the PAO_2, (and indirectly the PaO_2) against the reduced inspired partial pressure of oxygen (West, 1990). Depending upon the altitude, hyperventilation can raise the PAO_2 to viable levels, ensuring greater oxygen saturation of hemoglobin (Hgb). However, the PaO_2 is below the alveolar value because of the limited diffusion properties of the blood-gas barrier and the fact that at higher altitudes, oxygenation at rest, is occurring on the steep part of the oxyhemoglobin dissociation curve (West et al, 1983a, 1990). The oxygen pressure gradient between blood and tissue is most important for the final transfer of oxygen to the mitochondria, and this is reduced with altitude (Åstrand et al, 1986).

Altitude induced hypoxia and the lower PO_2 result in a decrease in the amount of oxygen available to perform exercise, and a reduction in maximal aerobic power of approximately 1% for every 100 meters above 1,500 meters (Buskirk, 1966). As altitude increases, the ventilation required to deliver the same amount of oxygen per unit of time must also increase (Banchero, 1987; Pugh et al, 1964).

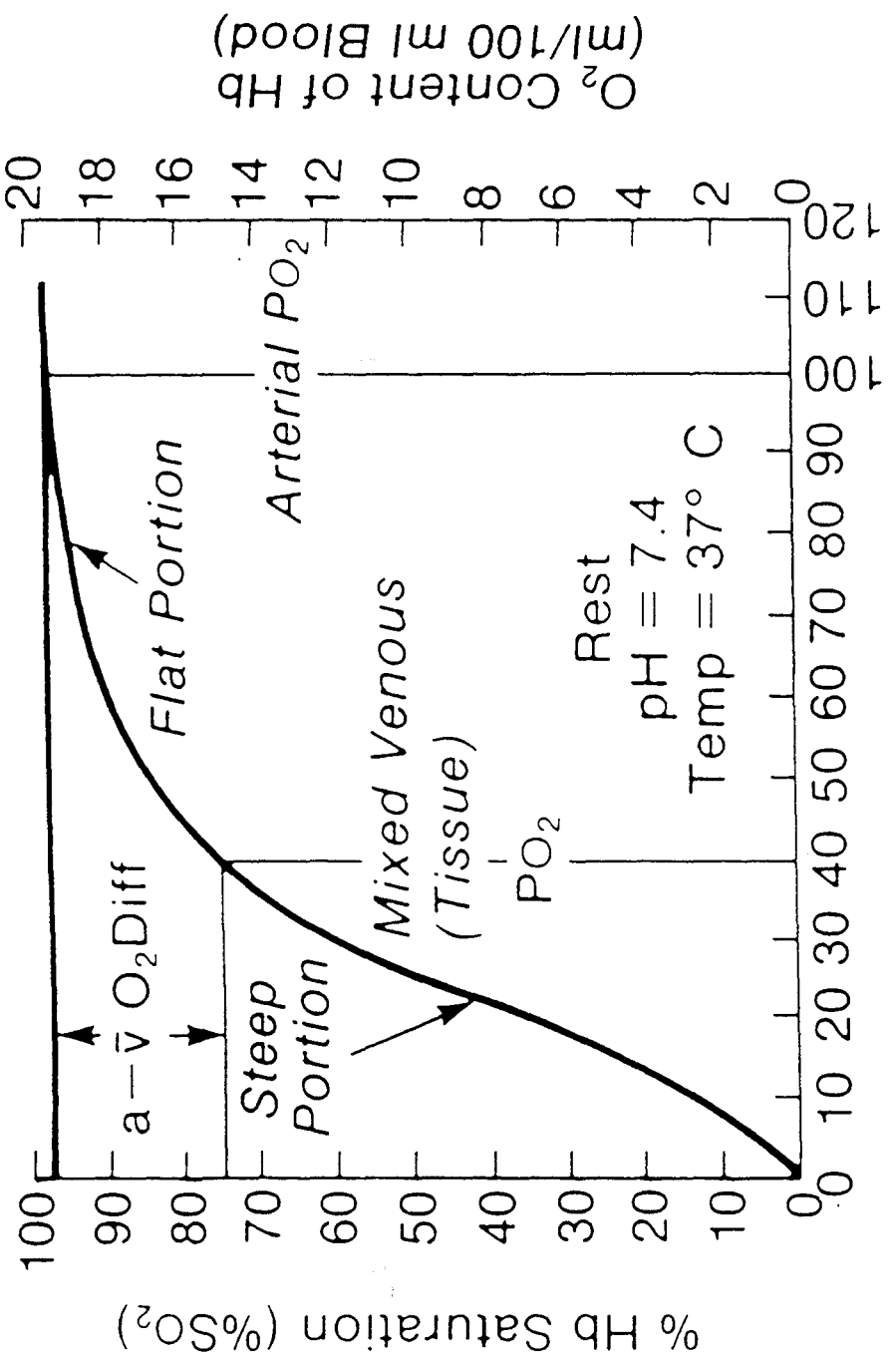
This hyperventilation results in decreased arterial carbon dioxide content ($CaCO_2$) and thus respiratory alkalosis. This response results in a leftward shift of the oxyhemoglobin dissociation curve, increasing the oxygen affinity of Hgb and assisting with loading of oxygen at the pulmonary capillary, but at the same time interfering with unloading in the peripheral capillaries (Cerretelli, 1976; West, 1990). The decreased arterial carbon dioxide partial pressure ($PaCO_2$) and alkalosis tends to attenuate the hypoxic ventilatory drive but after a few days, the pH of the arterial blood is returned to near normal, at which time the brakes on ventilation are reduced. Even so, with chronic exposure ventilation remains higher at altitude, especially during exercise, despite the inhibitory effects of hypocapnia and alkalosis (Sutton et al, 1990).

1.3 LEVEL OF ALTITUDE

During rest at altitude, relatively large decreases in PO_2 can be tolerated due to the sigmoidal shape of the oxyhemoglobin dissociation curve. For example, at an alveolar PO_2 of 50 mmHg (4,300 meters) SaO_2 has only been reduced to 85%, however as the level of altitude increases from this point, oxygen binding is now occurring on the steep part of the curve (Figure 1) and for that reason, a slight drop in PO_2 can markedly reduce the SaO_2 (Brooks et al, 1985).

During exercise, pulmonary diffusion is severely affected at altitude. The transit time in the pulmonary capillaries remains the same as at sea level (0.25 seconds), but reduced diffusion from impaired driving forces (lower PO_2) at altitude greatly affects SaO_2 and therefore $\dot{V}O_2max$ (Brooks et al, 1985).

Pulmonary ventilation at rest is not exaggerated unless the PAO_2 falls below 50-60 mmHg. A significant increase may be seen at such altitudes during exercise due to other factors such as an altered production of anaerobic metabolites or in individuals with very sensitive chemoreceptors (Faulkner et al, 1967). Therefore at low altitudes such as 2,000 meters (PAO_2= 67 mmHg), the hypoxic drive for increased ventilation is minimal (Faulkner, 1967).



(Fox et al, 1988)

Other acclimatization factors such as increased ventilation, and alkalemia, together with the shape of the oxyhemoglobin dissociation curve cause the resting and exercising saturations at low and moderate altitudes to drop only slightly from 95 to 80%. However, at high altitude despite the maintained PaO_2 during heavy exercise, the SaO_2 falls due to a lactacidosis (Hansen et al, 1967).

1.4 ACUTE EXPOSURE: EFFECTS ON EXERCISE

Acute exposure to altitude refers to exposures of up to 2 or 3 days. The lower PAO_2 results in an immediate decrease in $\dot{V}O_2max$, and maximal aerobic work capacity (Pugh et al, 1964; Shephard et al, 1988; West, 1990). During submaximal levels of exercise, $\dot{V}e$ is higher at altitude than when performing the same absolute power output at sea level (Pugh et al, 1964; West et al, 1983a). At low and moderate exercise intensities at altitude the exercising heart rate (HR) is also higher, compared to the same absolute exercise at sea level (Cerretelli, 1976; Escourrou et al, 1984; Jones et al, 1972; Klausen, 1966; Manchanda et al, 1975; McManus et al, 1974; Pugh et al, 1964; Schibye et al, 1988). The higher HR is a response to the lower stroke volume (SV) and maintains, (McManus et al, 1974; Rowell et al, 1986; Schibye et al, 1988; Wagner et al, 1980; West, 1990) or causes a

greater than normal cardiac output ($\dot{Q}c$) (Sutton et al, 1988). Lower intensities of exercise require, and result in, an increase in blood flow to the working muscles in order to compensate for the lower oxygen carrying capacity of the blood (Hartley et al, 1973; Jones et al, 1972; McManus et al, 1974; Rowell et al, 1986; Schibye et al, 1988; Wagner et al, 1980).

Blood lactate levels during submaximal exercise at altitude also tend to be higher than during exercise at the same power output at sea level, indicating an increased utilization of anaerobic metabolism to perform the work (Cerretelli, 1976; Jones et al, 1972; McManus et al, 1974; Wagner et al, 1980; Yoshida et al, 1989). There is also a marked reduction in the duration that a given absolute power output can be maintained. Although submaximal exercise under acute hypoxia results in relatively higher HR, $\dot{V}e$, and blood lactate concentrations, the maximal levels of $\dot{V}O_2$, HR, $\dot{V}e$, and blood lactate concentration which are reached are lower than those reached at sea level.

1.5 NATIVES OF ALTITUDE

There are many physiological differences between sea level and high altitude residents. Individuals living at altitude have demonstrated lower blood flow to exercising

muscles, higher maximum HR, lower $\dot{V}e$, and lower $\dot{Q}c$ (Hartley et al, 1967; Pugh et al, 1964) as compared to sea level dwellers exposed to the same elevations (Balke, 1964). Lower $\dot{V}e$ suggests that these individuals have greater pulmonary diffusion capacity resulting in higher arterial saturation (Sutton et al, 1990). Likewise, the cardiovascular system is able to perform the same amount of work with lower HR and resultant blood flow, compared to sea level natives (Sutton et al, 1990). At altitude, these native residents have lower SaO_2, but higher CaO_2 because of the lower $PACO_2$ (Moret et al, 1972).

When high altitude natives descend and perform exercise at sea level they have lower HR and decreased CaO_2 (Sime et al, 1971) with corresponding increased SV and $\dot{Q}c$ (Hartley et al, 1967; Sime et al, 1971) as compared to their sea level counterparts exercising at the same power output. However, the effects of these differences are controversial since Grover et al (1967) found no differences in exercise capacity and oxygen transport (SaO_2 and CaO_2) between the different residents performing at either sea level or altitude.

When performing at altitude, natives reach higher maximal oxygen consumption levels as compared to newcomers at the same altitude. Such differences demonstrate an

increased capacity for oxygen supply or oxygen utilization by the working muscle of altitude natives (Sutton et al, 1990). The majority of the superior performance of altitude natives is due to enhanced pulmonary diffusion and higher exercising $\dot{V}e$, possibly due to higher tissue diffusion (Sutton et al, 1990).

Natives of altitude tend to have higher levels of Hgb and hematocrit (Hct) resulting in an increased oxygen carrying capacity (Cerretelli, 1976). The partial pressure of oxygen at which the Hgb is half saturated (P-50) is higher in altitude residents demonstrating that the Hgb concentration restores CaO_2 (Sutton et al, 1990; Winslow et al, 1981). Reynafarje (1962) found higher muscle oxidative enzyme activities and myoglobin concentrations in altitude natives and concluded that the respiratory capacity of the muscle was higher.

Edwards (1936) first showed that acclimatized individuals had reduced peak blood lactate levels during exercise. These results were further supported by Pugh et al (1964), Cerretelli et al (1982), Hansen et al (1975), and West et al (1983b). Such results may reflect a reduced buffering capacity (Cerretelli et al, 1982) causing lower pH levels for a given production of lactic acid. Hypoxic exercise may also shift the fuel source, since others have

demonstrated an increase in fat utilization and decreased glycogenolysis, thereby reducing peak blood lactate concentrations (Sutton, 1977; Young et al, 1982).

Altitude natives have reduced blood bicarbonate levels (lower buffering capacity) therefore normalizing, or decreasing, the pH of the blood (Brooks et al, 1985). This results in a rightward shift of the oxyhemoglobin dissociation curve restoring the normal binding relationship between oxygen and hemoglobin and increasing the diffusion gradient. This increases tissue oxygenation since more oxygen is delivered at the same or higher oxygen tension (Brooks et al, 1985). The lower intracellular pH may inhibit anaerobic enzyme activity and limit energy flux in the glycolytic pathway thus reducing the accumulation of lactate (Cerretelli et al, 1990).

1.6 CHRONIC ACCLIMATIZATION

1.6.1 Respiratory Adaptations

As mentioned, an increase in $\dot{V}e$ is one of the most important adaptations to prolonged hypoxia. This response is immediate upon arrival at altitude, being more pronounced during the first few days, then stabilizing after about a week. Ventilation continues to increase over time at a given altitude, in spite of respiratory alkalosis which blunts the respiratory response to hypoxia. This

ventilatory response will further raise the partial pressure of oxygen and acclimatized individuals tend to exercise at the same oxygen requirements with a lower ventilation as compared to acutely exposed sea level residents (Pugh et al, 1964).

1.6.2 Blood Parameters

Acclimatization leads to higher red cell concentrations and Hgb levels (Cerretelli, 1976), resulting in increased oxygen carrying capacity. This response maintains the CaO_2 at normal or even above normal values, even when the PaO_2 and the SaO_2 levels are diminished (West, 1990). Winslow et al (1981) found the P-50 level to be significantly higher with prolonged exposure, and therefore demonstrating the increase in Hgb concentration is sufficient to restore sea level values of CaO_2 (Sutton et al, 1990).

The higher concentrations of Hgb and Hct (Reynafarje et al, 1959; Winslow et al, 1987, 1989) found after prolonged exposure to hypoxia are believed to be caused by a sustained increase in erythropoietin. Under conditions of maximal erythropoietic stimulation, such as altitude and exercise, the newly formed red blood cells will appear in circulation within 5 days (rather than the usual 7 days) (Lewis, 1989). This increase in Hgb concentration promoted

by the chronic hypoxic stimulus may more than compensate for the reduced percentages of oxygen saturation and normalize the CaO_2 (Cerretelli, 1976; Hansen et al, 1967). Arterial oxygen content decreases further with exercise and therefore more profound arterial hypoxemia constitutes an accentuated stimulus for transient erythropoietic production (Berglund, 1992).

Increases in Hgb levels have been explained as the result of a reduced plasma volume found during exposure to altitude. Increases in plasma volume found after training are thought to be induced by an increase in the activity of the renin-angiotensin-aldosterone system during exercise. A normal response is achieved within two weeks, and, at three weeks at altitude, the plasma volume may still be decreased (Dill et al, 1974) while erythropoietin continues to stimulate an increase in Hgb levels. Higher altitudes have shown longer time spans in order to normalize plasma volume, but, as noted, the red cell concentration remains high even after the plasma volume is stabilized.

With chronic hypoxia, there is also an increase in red blood cell 2,3-Diphosphyoglycerate (2,3-DPG) content. This increase shifts the oxyhemoglobin dissociation curve to the right which enhances the unloading of oxygen at the tissue level. An increase in 2,3-DPG red blood cell content

is an advantage at moderate to high altitudes where the steep part of the dissociation curve is affected. At this portion, slight decreases in PO_2 greatly diminish SaO_2. The increase in the red blood cell concentration of 2-3-DPG exaggerates this effect by shifting the curve to the right so that there is a lower SaO_2 (greater $(a\text{-}v)O_2$ difference) for the same PO_2, or a similar SaO_2 for higher PO_2s.

Oxyhemoglobin desaturation results in increases in adaptations that depend on oxygen delivery to peripheral tissues. With acclimatization, red cell mass does not increase until a PaO_2 below 65 mmHg (2,500 meters), which is also a point on the curve at which the oxygen saturation begins to fall rapidly (Levine et al, 1992). With adaptations such as increased Hct, the oxygen content of the blood drops less and therefore oxygen carrying capacity is higher (Hansen et al, 1967). Therefore the decrease in oxygen-hemoglobin affinity through adaptation is beneficial for tissue oxygen supply, because there is a facilitated unloading of oxygen from Hgb (Mairburl et al, 1986). Increases in the 50% saturation level after prolonged exposure are also partly due to increases in red cell 2,3-DPG which influences a rightward shift in the dissociation curve to increase $(a\text{-}v)O_2$ difference at lower PO_2s.

The increase in Hgb concentration and the shift in the operational range to the steeper slope of the oxyhemoglobin dissociation curve provide major contributions to the gradually increased oxygen delivery within the body at altitude (Åstrand et al, 1986). The gradual decline in $\dot{Q}c$ (Sutton et al, 1988) during prolonged exposure to a low PO_2 can be partially explained by this concomitant rise in the oxygen combining capacity of the blood (Åstrand et al, 1986). The increase in CaO_2 which results from both ventilatory acclimatization and stimulated erythropoiesis helps to maintain oxygen delivery (Wolfel et al, 1989).

1.6.3 Skeletal Muscle Adaptations

There are many adaptations which may occur at the muscle tissue level in order to improve exercise capacity. Earlier studies have shown increases in muscle capillarity (Banchero, 1975), increased mitochondrial volume, and increases in myoglobin concentration (Reynafarje, 1962; Terrados et al, 1990; Valdivia, 1958). These intramuscular changes determine the oxidative capacity of the tissue. Increased capillarization reduces the diffusion distance and enhances the transportation of oxygen from blood to the mitochondria while increases in myoglobin enhances the storage capacity of oxygen and the increase in mitochondrial volume reduces the dependence on anaerobic ATP production

(Åstrand et al, 1986). However, most of these reported adaptations have come from animal studies and are generally not supported by similar findings in humans (Boutellier et al, 1983; Cassin et al, 1971; Mizuno et al, 1985; Terrados et al, 1985).

Evidence from humans acclimatized to chronic hypoxia have shown controversial results. With severe hypoxia, it has been shown (Green et al., 1989; Bigard et al., 1991; Boutellier et al., 1983; Hoppeler et al., 1990; Howald et al., 1990; MacDougall et al., 1991), that reductions in oxidative potential occur, although there is an increase in capillary density. Since the absolute number of capillaries was unchanged, however, these results were apparently caused by reduction in fiber area rather than to growth of new capillaries. Theoretically, this could be considered an advantage since it results in an enhancement in the diffusion of oxygen from capillaries to muscle fibers (Banchero, 1987; Green et al, 1989). Hoppeler et al (1990) showed reduced mitochondrial density in muscle samples which may again be the result of muscle loss and corresponding reductions in intra-muscular structures.

Conversely, Reynafarje (1962) found higher muscle oxidative enzyme activities in acclimatized individuals and concluded that the respiratory capacity of the muscle was higher. This researcher added that the glycolytic enzymes,

which decreased, were not significantly involved in the adaptive process to high altitude. Additionally, research from chronic hypoxic exposure in which individuals maintained regular physical activity levels have shown substantial elevations in the activity of mitochondrial enzymes (Saltin et al, 1983; Terblanche et al, 1983) indicative of a higher respiratory capacity.

1.7 CHRONIC HYPOXIA: EFFECTS ON EXERCISE

Chronic hypoxia refers to an exposure to altitude of more than 2 or 3 days. Acute exposure to altitude results in a functional impairment, but chronic exposure improves the performances observed under such conditions. Chronic exposure to hypoxia combined with physical training results in an improvement in the ability to perform submaximal work at altitude (Levine et al, 1992; Maher et al, 1974; Terrados et al, 1988). Such prolonged stays are accompanied by drawbacks, most notably a loss of muscle tissue (Hoppeler et al, 1990; Howald et al, 1990; MacDougall et al, 1991).

Increasing levels of altitude result in reduced oxygen delivery to the working muscle. However, as mentioned, individuals acclimatized to altitude adapt with changes such as increased Hgb and Hct which allow for an enhancement of the blood's oxygen carrying capacity. These acclimatized individuals also tend to have an increased red

blood cell content of 2,3-DPG which promotes oxy-hemoglobin dissociation and tissue oxygenation (Alexander et al, 1967; Grover et al, 1976). Yet, even with such adaptations $\dot{V}O_2max$ remains similar to that which occurs with acute exposure.

Prolonged exposure to altitude results in reduced SV with a higher HR at light and moderate work intensities, therefore maintaining $\dot{Q}c$ (Cerretelli, 1976; Pugh et al, 1964; Reeves et al, 1987; Sawka et al, 1989). Maximal $\dot{Q}c$ is however known to decrease (Cerretelli, 1976; MacDougall et al, 1976; Pugh et al, 1964; Reeves et al, 1987; Saltin, 1968), but Cerretelli (1980) states that the reduction in maximal flow is not enough to decrease $\dot{V}O_2max$ to its observed levels.

Submaximal and maximal blood lactate levels are lower during exercise after chronic altitude exposure (Green et al, 1989; Levine et al, 1992; Sutton et al, 1988). One explanation is that the lactate dehydrogenase enzymes could shift to function as mitochondrial isoenzyme within the muscle, favouring pyruvate oxidation and decreasing lactate production (Sutton et al, 1990). Reduced production of lactic acid may also result from a lower intracellular pH which inhibits anaerobic enzymes of the glycolytic pathway (Cerretelli et al, 1990). Impaired lactate production and lactate efflux from muscle to blood during exercise may also

be the result of respiratory alkalosis (decreased PCO_2) and metabolic acidosis (decreased HCO_3) (Sutton et al, 1981).

Overall, prolonged exposure to altitude improves maximal aerobic capacity (MAC), yet there is no increase in $\dot{V}O_2max$. Such developments are suggestive of muscular adaptations to hypoxia since $\dot{V}O_2max$ is known to be primarily limited by oxygen delivery rather than oxygen utilization.

1.8 DIFFERENTIAL ADAPTATIONS IN $\dot{V}O_2max$ AND MAC

It is well known that the local aerobic capacity of skeletal muscle is improved in response to endurance training and attributes such as increased oxidative capacity and capillary density play a significant role (Sundberg et al, 1993). Hypoxia can act as an additional stimulus for local muscular adaptation to endurance training. Enhancements such as oxidative enzyme content and the proportion of type IIA fibers increase the capacity for oxygen utilization, thus augmenting aerobic performance capacity (MAC), but not $\dot{V}O_2max$ (Sundberg et al, 1993).

Increases in Hgb concentration have been shown to enhance aerobic power, while increases in capillary density, mitochondrial number, tissue myoglobin concentration, and 2,3-DPG affect peripheral uptake of oxygen by the exercising muscle and therefore aerobic capacity (Levine et al, 1992;

Mairburl et al, 1986; Reynafarje et al, 1975; Terrados et al, 1990).

As can be seen from the above, the way in which performance is measured can greatly affect the results and conclusions of the research. Studies that examine only $\dot{V}O_2max$ as an index of performance may miss important adaptations such as changes in substrate utilization (Brooks, 1991; Young et al, 1982), or buffering capacity of the skeletal muscle (Mizuno et al, 1990) that may thus improve aerobic capacity and therefore should be assessed by changes in MAC and not $\dot{V}O_2max$ alone.

1.9 ALTITUDE TRAINING AND PERFORMANCE AT SEA LEVEL

Early investigations of training at altitude demonstrated improved performance and increased maximal aerobic power upon return to sea level (Balke et al, 1964, 1965; Faulkner et al, 1967), however these findings may have been the result of a significant training effect having occurred at altitude. It was noted that the subjects had not achieved a plateau in performance prior to altitude training and enhancements were influenced by further training increases. Some investigators have observed the same improvements with well trained athletes (Daniels et al, 1970; Dill et al, 1971; Klausen, 1966) but these results

have been questioned due to the failure to have appropriate control groups.

More recent studies have demonstrated improvements in work capacity, lower exercising blood lactate concentrations during submaximal exercise, increased capillarization and reduced glycolytic capacity with training at moderate altitude as compared to training of the same intensity at sea level (Mairbaurl et al, 1986; Terrados et al, 1988). Altitude training has been shown to increase the activities of oxidative enzymes and to improve endurance capacity as compared to the same training performed at sea level (Terblanche et al, 1983; Terrados et al, 1990). Still others have shown skeletal muscle adaptations but no improvements of aerobic power upon return to sea level (Levine et al, in press).

The above results would lead one to assume that altitude training may improve performance by a number of mechanisms affecting both oxygen delivery and extraction, as well as substrate utilization (Young et al, 1982) and skeletal muscle buffering capacity (Mizuno et al, 1990). Whether or not hypoxia (altitude) in combination with exercise results in a greater stimulus for enzymatic and structural adaptations than that which occurs with normoxic exercise remains controversial (Terrados et al, 1990). Evidence tends to support enhancements of aerobic

mitochondrial enzyme activities, though this effect does not clearly improve performance upon return to sea level (Levine et al, in press).

1.10 PURPOSE

The purpose of the present study was to determine whether or not the combination of normobaric hypoxia and exercise training would enhance adaptations in skeletal muscle over and above that which occurs with the same amount of training under normoxic conditions. Also investigated was the effect of such training conditions on performance as assessed by measurements of $\dot{V}O_2max$ and MAC.

CHAPTER II

2.1 INTRODUCTION

The progressive decline in maximal oxygen consumption ($\dot{V}O_2max$) and maximal aerobic capacity (MAC) with ascent to altitude has been well documented (Adams et al, 1975; Anderson et al, 1985; Buskirk et al, 1966, 1967; Cerretelli et al, 1976; Klausen et al, 1970; Pugh et al, 1964; West et al, 1983a). It is also known that physiological adaptations which occur due to altitude training and acclimatization improve performance at altitude, but the controversial question is whether or not such adaptations are able to improve performance upon return to sea level. Despite minimal supportive evidence, coaches and athletes continue to sojourn to altitude for periodic training sessions in preparation for sea level performance.

Earlier investigations that demonstrated altitude training to enhance aerobic power upon return to sea level have been criticized in that they may simply have been the result of a significant training effect in previously unconditioned individuals (Balke et al, 1964, 1965; Faulkner et al, 1967, 1968; Klausen 1966). Further investigations with well trained athletes have also demonstrated such

improvements in performance upon return to sea level (Daniels et al, 1970; Dill et al, 1971) although these findings have also been questioned due to the lack of appropriate control groups. More recently, Terrados et al (1988) demonstrated that training under hypoxia improved sea level performance over and above the same training performed at sea level. These findings demonstrate the importance of such interrelated factors as the length of the sojourn, the level of the altitude, the type of training, and the intensity of training.

Much research has demonstrated acclimatization factors which improve performance at altitude. Altitude training may improve performance by a number of mechanisms. Higher levels of Hgb and Hct improves oxygen delivery (Hansen et al, 1967) while increased capillary density, mitochondrial number, tissue myoglobin concentration and red blood cell 2,3-DPG content enhances oxygen extraction (Reynafarje et al, 1975; Terrados et al, 1990). Acclimatization has resulted in increased work capacity (Maher et al, 1974), and lower levels of blood lactate (Cerretelli, 1982; Edwards, 1936; Hansen et al, 1975; Pugh et al, 1964; West, 1983) when performing the same absolute exercise intensity; however, there are no improvements in $\dot{V}O_2max$ at altitude. This increase in work capacity without

increased $\dot{V}O_2max$ would suggest that with acclimatization adaptations may have occurred at the muscle level.

Others have investigated and found that acclimatization alone (i.e., not combined with exercise training) does not result in these adaptations in skeletal muscle. Chronic exposure to extreme altitude results in muscle atrophy and concomitant misleading increases in capillary and mitochondrial densities (Bigard et al, 1991; Boutellier et al, 1983; Green et al, 1989; Hoppeler et al, 1990), as well as decreased fiber area for shorter oxygen diffusion distances (Banchero, 1987; Green et al, 1989). Muscle atrophy resulting from severe hypoxic exposure may result in reduced mitochondrial density and mitochondrial enzyme activity capacity (Green et al, 1989; Hoppeler et al, 1990).

Enhanced sea level performance may therefore be the result of a combined stimulus of hypoxia and exercise. Terrados et al (1990) found that exercising under hypoxic conditions leads to increases in oxidative enzyme activity and myoglobin when compared to the same training under normoxia. In contrast, others have found the oxidative enzyme activities to decrease during hypoxic training (Boutellier et al, 1984). When training at altitude, at the same absolute intensity as at sea level, evidence supports a

further increase in mitochondrial enzyme activities, though this has not necessarily shown improved performance at sea level (Terrados et al, 1990).

The purpose of this study was to determine whether or not the combination of normobaric hypoxia and exercise training would enhance adaptations in skeletal muscle over and above that which occurs with the same amount of training under normoxic conditions.

2.2 METHODS

A pilot study was conducted to determine the optimum hypoxic condition for training. Five healthy subjects (3 males and 2 females) performed progressively increasing cycle ergometer tests to fatigue under four different inspired fractional concentrations of oxygen (FIO_2): 20.93%, 14.0%, 12.5%, and 11.0%. Main effects for increasing levels of hypoxia demonstrated reduced $\dot{V}O_2max$ and HR_{max} as compared to normoxia ($p< 0.05$). At a given absolute workload (60% of maximum power output) there was also a main effect for the level of hypoxia since $\dot{V}O_2$ was lower and blood lactate concentrations, $\dot{V}e$, and HR were higher with decreasing FIO_2 ($p< 0.05$). Tukey's post hoc procedures indicated the majority of the significant differences in $\dot{V}O_2$, $\dot{V}e$, HR and blood lactate concentrations to occur when breathing an FIO_2 below 14.0%.

An inspired fractional concentration of oxygen (FIO_2) of 13.5% was selected since it represented the lowest FIO_2 in which subjects were able to perform 30 minutes of continuous activity at 75% of maximal normoxic power output. From the pilot study, it was found that with an FIO_2 of 14.0% subjects were still able to achieve the same absolute $\dot{V}O_2$ at 75% of maximal power output. Since subjects were able

to maintain and complete exercise sessions at this FIO_2, 13.5% was used for the unilateral training level in the present research. An FIO_2 of 13.5% results in an inspired PO_2 of 103 mmHg which corresponds to an altitude of ~3,292 meters.

2.2.1 Subjects

Ten healthy male subjects, 19-25 years of age, consented to participate in the study. Subjects were physically active but had never undergone previous endurance training and were instructed to maintain current activity levels and dietary habits during the course of the study. Subjects were well informed as to the purpose, procedures and any possible risks associated with the study as approved by the Presidents' Committee on the Ethics of Research on Human Subjects (Appendix B). All were familiarized with the equipment, testing procedures and unilateral cycling before the onset of the study.

2.2.2 Procedures

2.2.2.1 Performance Measures

Unilateral exercise tests before and after training were performed on a cycle ergometer under normoxic

conditions. The foot of the exercising limb was held securely to the pedal with toe clips and tape while the non-exercising limb rested in a standard position on a chair placed to the side of the cycle.

Subjects performed unilateral maximal progressive cycle ergometer exercise tests on an electrically braked cycle (Erich Jaeger) that allowed a given power output to be maintained at pedal frequencies between 60 and 90 revolutions per minute (rpm). The initial power output was 75 W and was increased every three minutes (15 or 30 W) until maximum, as determined by the following criteria: 1) a plateau in $\dot{V}O_2$, 2) a RER > 1.15, 3) failure to maintain pedal frequency above 60 rpm, and/or 4) volitional fatigue. Peak values attained during the test were considered to be "maximum" and total test time was recorded to the nearest 0.01 seconds. The highest intensity performed for one minute was considered to be maximum power output.

For each test, $\dot{V}O_2$, $\dot{V}e$, HR, blood lactate, RER, power output and test duration were continually monitored. Expired gas was analyzed by a computerized open circuit system (Ametek S-3A/1 Oxygen Analyzer; Hewlett Packard 78356A Carbon Dioxide Analyzer) with calculations being made every 30 s. HR was monitored by a three lead ECG and finger tip blood samples were taken every minute to assess blood

lactate concentration (Yellow Springs YSI Model 23L Lactate Analyzer). All analyzers were calibrated before and after each test.

Subjects also performed a unilateral MAC test to fatigue at a power output corresponding to 95% of each leg's pre-training maximal power output. Fatigue was determined by an inability to maintain 60 rpm, and/or volitional exhaustion and endurance was expressed as the time to fatigue to the nearest 0.01 seconds. All exercise measures were continuously monitored and finger-tip blood samples were taken at ten minute intervals and/or at exhaustion.

2.2.2.2 Training Program

Subjects acted as their own controls and were randomly assigned to one of two groups. One group trained the left leg under normoxia and the right leg under hypoxia (FIO_2= 13.5%); the other group trained the right leg under normoxia and the left leg under hypoxia. The hypoxic condition was achieved by utilizing a 350 L Tissot gasometer and an electrically controlled system for diluting ambient air by bleeding nitrogen into it at a controlled rate. An oxygen analyzer was used to monitor the inspired oxygen concentration and a variation of less than 0.2% was maintained during the training. When subjects trained under

the hypoxic condition they breathed the gas mixture through a standard Rudolph valve. Before the mixture was drawn (by vacuum pump) into the tissot tank, the ambient air was bubbled through a smaller tank filled with water to be moisturized. Subjects also breathed through the same system when breathing ambient air for the pre- and post-training performance tests.

The endurance training consisted of unilateral cycle ergometry exercise three times per week for 8 weeks. The absolute intensity was the same and selected on the basis of the lowest value recorded for the two legs. Initially during each training session, the subjects cycled continuously with each leg for 30 minutes at an intensity corresponding to 75% of the weakest leg's pre-training maximal power output. As the subjects gradually adapted to the training the power output was increased (5% of maximal power output) in order to provide progressive overload. After six weeks of training, the final two weeks consisted of 5, 3 min intervals at 100% of one-legged pre-training maximal power output, with 3 min recovery intervals, followed by 10 min of continuous cycling at the sixth week continuous training intensity. The order of the training legs were alternated from one session to the next.

2.2.2.3 Muscle Biopsies

Needle biopsies were extracted from the vastus lateralis of the right leg before the training period and from both legs after the training period using the Bergström technique (Bergström, 1962) and applying "suction" with a 50 ml syringe. On each occasion 2 biopsy samples were taken. The first sample was divided for electron microscopy and histochemistry. The second sample was immediately frozen in liquid nitrogen for subsequent biochemical analysis.

Histochemistry. Histochemical samples were frozen in isopentane precooled with liquid nitrogen. Cryostat sections (7 um) were mounted on glass slides which were then stained for myofibrillar ATPase activity following pre-incubation at pH 4.3, 4.6, and 10.0 (Padykula et al, 1955) to distinguish type I and II fibers, as well as to determine fiber area. To determine the number of capillaries, the cross-sections were stained by haematoxylin and eosin. The histochemical slides were photographed under a light microscope (American Optical Series 20). For each slide three photographic fields were randomly selected and photographed. The slide was projected onto a 144 square grid (Weibel, 1979), causing a surface area of 1406 cm^2 using a Recordak (MPE-1 Film Reader) projector and the number of visible capillaries were expressed per mm^2 as well

as per fiber. Cross-sectional area for type I and type II fibers was measured by a custom-made computerized digitizer for an average of 100-150 fibers of each type per biopsy. Percent fiber type distribution was estimated by counting an average of 250-300 fibers per biopsy.

Electron Microscopy. This tissue was immediately fixed in 2% glutaraldehyde, washed in 0.2 M cacodylate buffer, post-fixed in osmium tetraoxide, dried in ethanol and imbedded in epoxyresin. Serial ultrathin sections were made at a slight oblique (75°) angle to the fibers and mounted on copper/rhodian grids. These sections were photographed at approximately 50,000 X magnification under a Philips EM 301. Where possible, 50 fibers were randomly selected per biopsy and for each a photographic field for the interior of each fiber was randomly selected and photographed. Stereological analysis was performed on each micrograph by means of a 168 point shortline test system (Weibel, 1979) according to the method as previously described by Hoppeler et al, (1973). For each biopsy volume densities were calculated for myofibrils, interior mitochondria, lipid and cytoplasm.

Biochemistry. The second muscle sample was immediately frozen in liquid nitrogen and stored at -80 °C for biochemical analysis at the University of Waterloo. All enzyme analyses of specimens from before and after training

were performed on the same day. The activities of the different enzymes were measured by flourometric methods after the muscle specimen was dried, weighed and homogenized in 50% glycerol, 20mM sodium phosphate buffer (pH =7.4), 5mM B-mercaptoethanol, 0.5 mM ethylenediaminetetraacetic acid (EDTA) and 2% bovine serum albumin (Essen et al, 1975: Lowry et al, 1972). The following enzymes were assessed: Citrate Synthase (CS), Phosphofructokinase (PFK), and Succinate Dehydrogenase (SDH). Values are expressed as either umol or mmol per hour per gram of protein ($umol \cdot hr^{-1} \cdot g^{-1}$ / $mmol \cdot hr^{-1} \cdot g^{-1}$)

2.2.3 Statistical Analyses

All results are presented as means ± SD. A two factor (condition x training) repeated measure of analysis was used to assess significance which was accepted at $p < 0.05$. Tukey's post hoc procedures were utilized for further analyses.

2.3 RESULTS

2.3.1 Performance Measures.

2.3.1.1 Maximal Oxygen Uptake

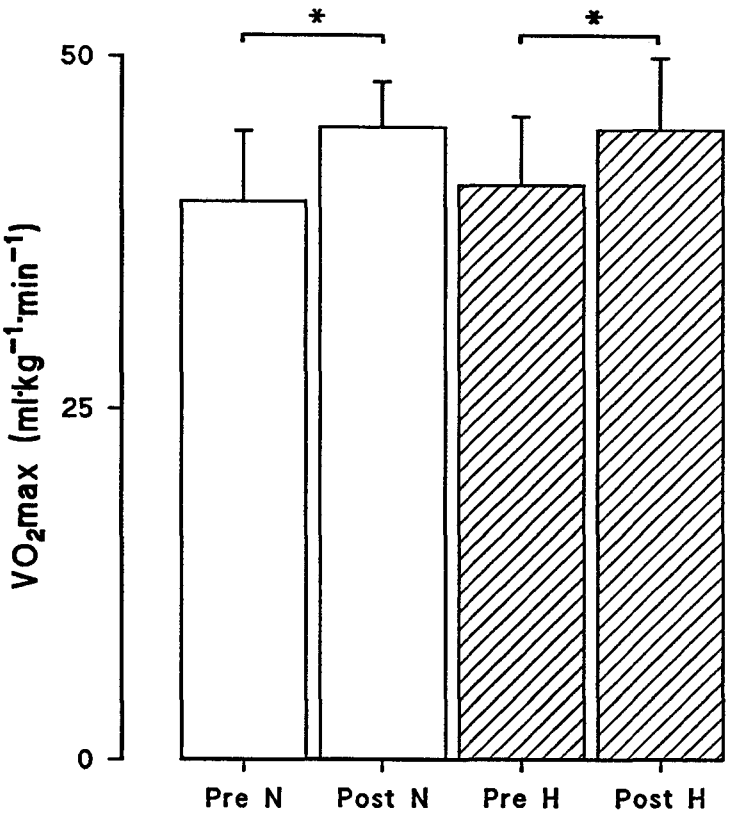
$\dot{V}O_2max$ for unilateral cycling is shown in Table 2 and Figure 2 (top) for both pre- and post-training tests. There was no difference between the pre-training value for the normoxically-trained legs (39.7 ± 4.9 $ml \cdot kg^{-1} \cdot min^{-1}$) and the hypoxically-trained legs (40.8 ± 4.8 $ml \cdot kg^{-1} \cdot min^{-1}$). Following training $\dot{V}O_2max$ increased significantly ($p < 0.05$) in both legs (13.0% in the normoxically-trained leg and 9.5% in the hypoxically-trained leg) with no difference between conditions.

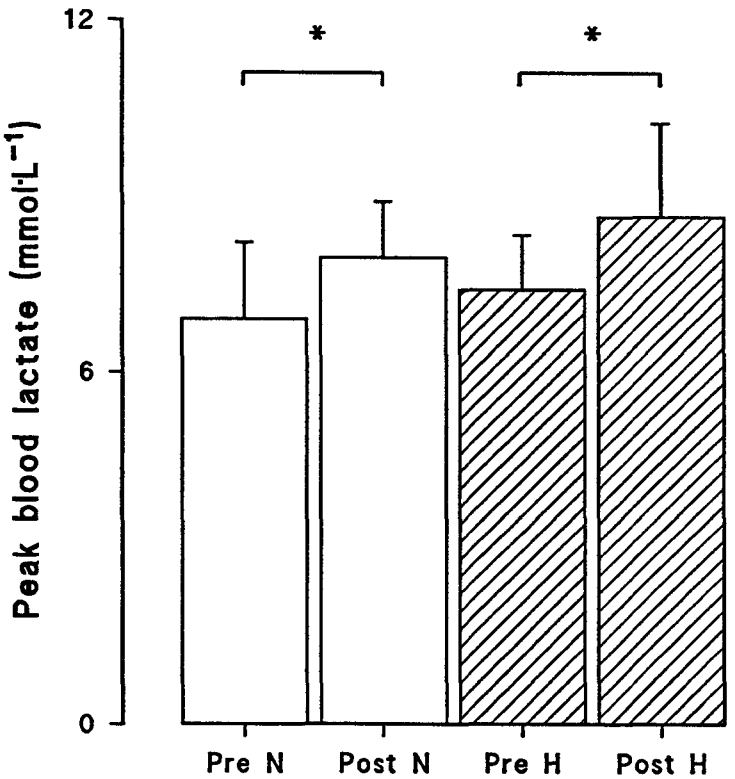
There were no significant differences in peak HR or peak $\dot{V}e$ between the tests for each leg pre- or post-training (Table 2). There were, however, significant increases in peak blood lactate following training ($p < 0.05$). Peak blood lactate was 15.0% higher following the normoxically-trained leg condition (6.9 to 7.9 $mmol \cdot L^{-1}$) and 16.8% higher following the hypoxically-trained leg condition (7.4 to 8.6 $mmol \cdot L^{-1}$) but there was no difference between conditions (Figure 2, bottom; Table 2).

TABLE 2: Peak values for unilateral maximal progressive cycle ergometry tests. Data given are pre- and post-training for both the normoxically- and hypoxically-trained legs. Values given are means ± (SD).

	NORMOXIA		HYPOXIA	
	PRE	POST	PRE	POST
POWER OUTPUT (W)	166.5 (16.5)	186.5* (27.5)	171.5 (18.4)	184.5* (25.4)
$\dot{V}O_2$ max ($ml \cdot kg^{-1} min^{-1}$)	39.7 (4.9)	44.9* (3.3)	40.8 (4.8)	44.7* (5.1)
HR (bpm)	175 (10.8)	178 (11.7)	177 (11.1)	176 (12.1)
$\dot{V}e$ ($L \cdot min^{-1}$, STPD)	106.2 (12.0)	115.8 (17.5)	106.3 (13.5)	115.3 (14.4)
BLOOD LACTATE ($mmol \cdot L^{-1}$)	6.9 (1.3)	7.9* (1.0)	7.4 (0.9)	8.6* (1.6)
MAC (min)	13.0 (4.9)	65.0* (19.4)	10.4 (4.8)	63.5* (17.6)

* indicates $p < 0.05$; main effect for training


*
*
50
25
0
VO_2max ($ml \cdot kg^{-1} \cdot min^{-1}$)
Pre N
Post N
Pre H
Post H


*
*
12
6
0
Peak blood lactate ($mmol \cdot L^{-1}$)
Pre N
Post N
Pre H
Post H

2.3.1.2. Maximal Power Output

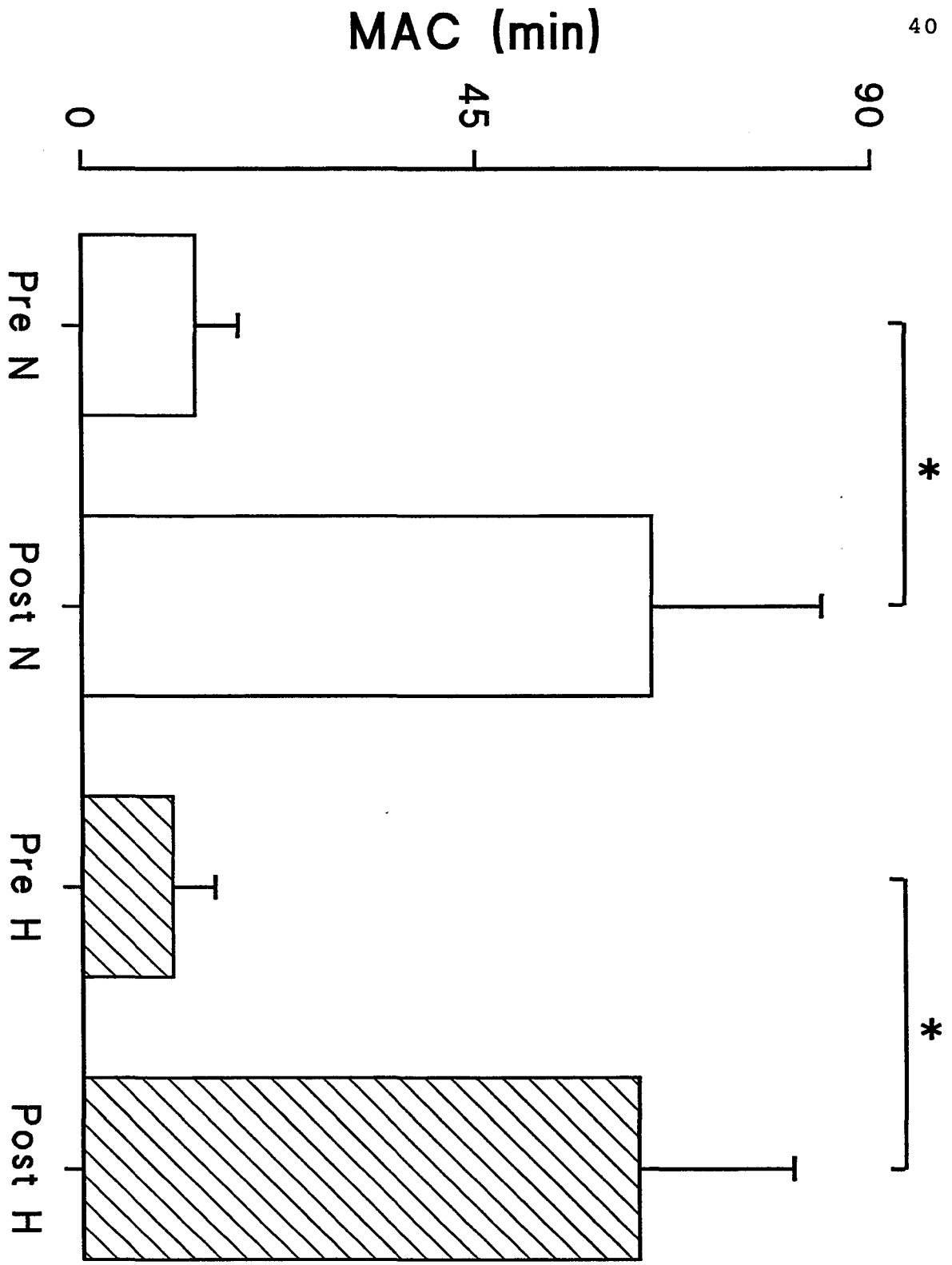
Post-training maximal power outputs increased significantly ($p < 0.05$) in both legs (12.0% in the normoxically-trained leg, and 7.6% in the hypoxically-trained leg) but there was no difference between training conditions pre- or post-training (Table 2).

2.3.1.3 Maximal Aerobic Capacity

Significant increases in maximal aerobic capacity were found for both legs following training ($p < 0.05$). Time to fatigue at a power output corresponding to 95% of the pre-training maximal power output increased from 13.0 (± 4.9) to 65.0 (± 19.4) minutes in the normoxically-trained leg and from 10.4 (± 4.8) to 63.5 (± 17.6) minutes in the hypoxically-trained leg (Figure 3, Table 2). There was no significant difference between conditions pre- or post-training.

2.3.2 Physiological Measures at the Same Absolute Submaximal Power Output

When data were examined at the same absolute power output (75% of pre-training maximum) it was found that there was a main effect for training on $\dot{V}e$ since it was significantly ($p < 0.05$) lower post-training when performing the same absolute amount of work with the normoxically-


MAC (min)
0
45
90
Pre N
Post N
Pre H
Post H
*
*

(56.7 ± 8.7 to 47.5 ± 3.7 $L \cdot min^{-1}$) and the hypoxically- (54.2 ± 10.2 to 50.3 ± 6.3 $L \cdot min^{-1}$) trained legs. There was no difference between training conditions (Table 3).

There was a main effect for training on HR, with post-training being significantly ($p < 0.05$) lower in both legs (from 151 ± 12 to 138 ± 12 bpm in the normoxically-trained leg, and from 150 ± 10 to 139 ± 17 bpm in the hypoxically trained leg), but no differences between conditions pre- or post-training (Table 3).

There were no differences in blood lactate concentration between either condition, pre- or post-training (Table 3).

2.3.3 Biochemical Data

There was a significant ($p < 0.05$) interaction between condition and training for citrate synthase (CS) activity (Figure 7, Table 6). CS activity was 42% higher in the normoxically-trained leg, and 67% higher in the hypoxically-trained leg. This increase was significantly greater ($p < 0.05$) in the hypoxically-trained leg. SDH (Figure 7, Table 6) and PFK (Figure 7, Table 6) activities showed a main effect for training and increased significantly from 2.31 ± 0.52 to 2.82 ± 0.46 $mmol \cdot hr^{-1} \cdot g^{-1}$ and 115.84 ± 24.62 to 177.95 ± 46.69 $umol \cdot hr^{-1} \cdot g^{-1}$ in the

normoxically-trained leg and from 2.31 ± 0.52 to 3.06 ± 0.51 $mmol \cdot hr^{-1} \cdot g^{-1}$ and 115.84 ± 24.62 to 188.51 ± 23.40 $umol \cdot hr^{-1} \cdot g^{-1}$ in the hypoxically-trained leg, respectively ($p < 0.05$). PFK and SDH did not show any differences in their activity levels between training conditions.

2.3.4 Morphometric Data

2.3.4.1 Capillary/Fiber Ratio and Capillary Density

Capillary/fiber ratios and capillary densities are presented in Table 4. There were no differences in the capillary/fiber ratios for either leg under either training condition. Mean values for capillary/fiber ratios were 15.6% and 26.2% higher in the normoxically- and hypoxically-trained leg respectively, following training (Figure 4, Table 4), but these differences were not statistically significant. Capillary density was not different among conditions or between trained legs (Figure 4, Table 4). Mean values for capillary density were 5.3% and 9.8% higher in the normoxically- and hypoxically-trained leg, respectively but these differences were not statistically significant.

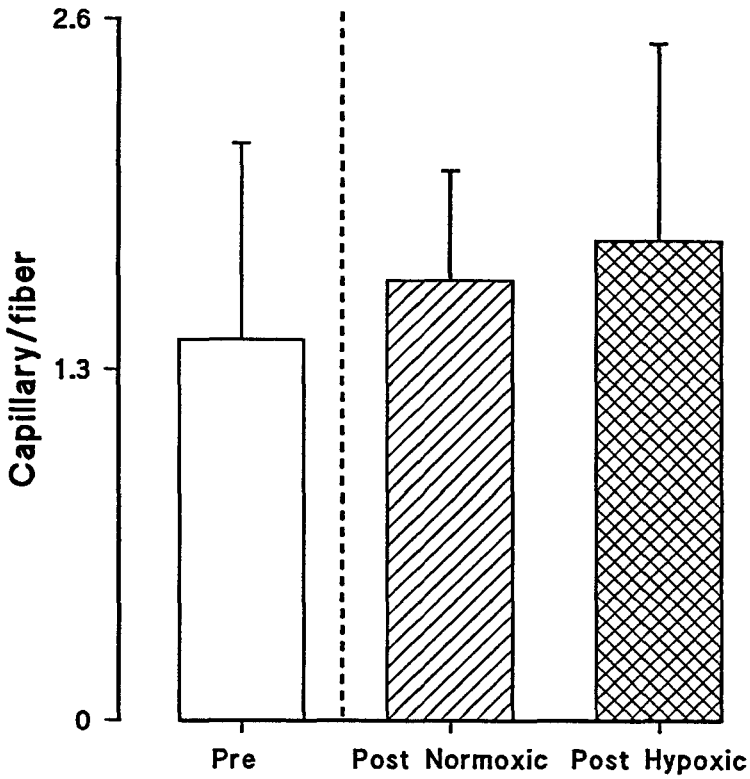
TABLE 3: Physiological measures at the same absolute power output (75% pre-training maximum). Data given are pre- and post-training for both the normoxically- and hypoxically-trained legs. Values given are means ± (SD).

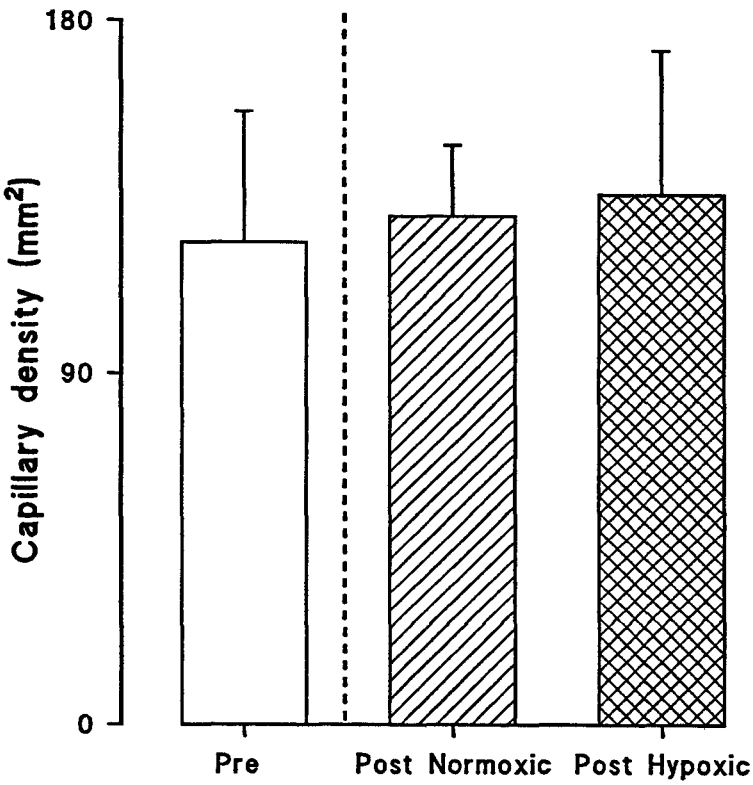
	NORMOXIA		HYPOXIA	
	PRE	POST	PRE	POST
$\dot{V}O_2$ ($ml \cdot kg^{-1} \cdot min^{-1}$)	27.9 (2.9)	27.1 (3.9)	28.0 (3.7)	28.2 (3.1)
HR (bpm)	151 (11.7)	138* (11.7)	150 (10.5)	139* (16.8)
$\dot{V}e$ ($L \cdot min^{-1}$, STPD)	56.7 (8.7)	47.5* (3.7)	54.2 (10.2)	50.3* (6.3)
BLOOD LACTATE ($mmol \cdot L^{-1}$)	3.0 (0.5)	3.4 (0.6)	3.2 (0.7)	3.4 (1.0)

TABLE 4: Morphometric data. Data given are pre- and post-training for both the normoxically- and hypoxically-trained legs. Values given are means ± (SD).

	PRE TRAINING	POST NORMOXIA	POST HYPOXIA
CAP/FIBER	1.4 (0.7)	1.6 (0.4)	1.8 (0.7)
CAPILLARY DENSITY (per mm^2)	123.0 (33.4)	129.6 (18.0)	135.0 (37.1)
FIBER AREA			
Type I (um^2)	59.1 (18.6)	60.9 (7.1)	58.9 (15.9)
Type II (um^2)	69.5 (17.2)	86.9 (24.5)	80.4 (28.3)
FIBER TYPE (% Type I)	40.6 (11.0)	41.5 (15.3)	44.9 (10.5)

* indicates $p < 0.05$; main effect for training


2.6
1.3
0
Capillary/fiber
Pre
Post Normoxic
Post Hypoxic


180
90
0
Capillary density (mm²)
Pre
Post Normoxic
Post Hypoxic

2.3.4.2 Fiber Area

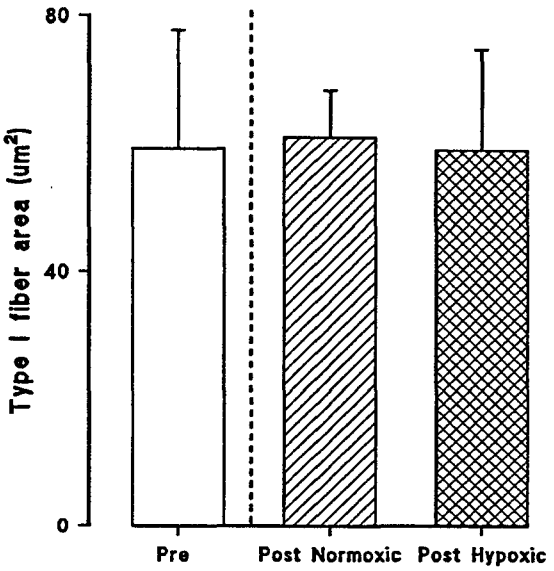
There were no differences in the cross sectional areas of the type I (Figure 5, top; Table 4) or type II (Figure 5, middle; Table 4) muscle fibers with either condition or training.

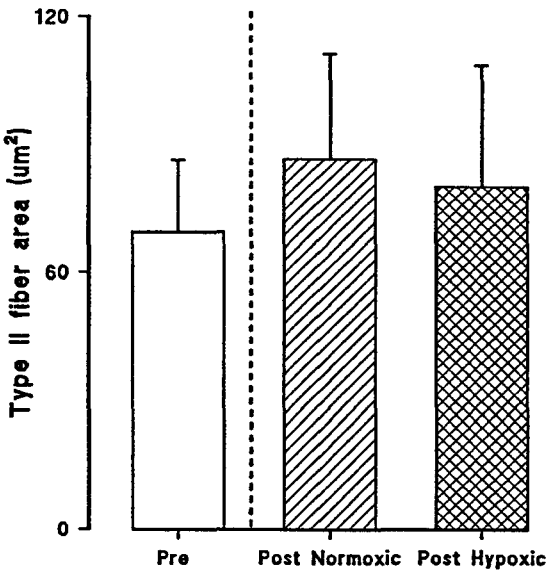
2.3.4.3 Fiber Type

There was no difference in fiber type distribution between the two conditions or after training. The mean distribution of type I fibers varied from 40.6% to 44.9% in the 3 samples but differences were not statistically significant (Figure 5, Table 4).

2.3.4.4 Muscle Ultrastructure

Muscle ultrastructural data are presented in Table 5. There were no differences in mitochondrial density for either leg under either condition. Following training, mean values were 5.0% higher in the normoxically-trained leg and 9.0% higher in the hypoxically-trained leg (Figure 6), but these differences were not statistically significant.


80
40
0
Type I fiber area (um²)
Pre
Post Normoxic
Post Hypoxic


120
60
0
Type II fiber area (um²)
Pre
Post Normoxic
Post Hypoxic

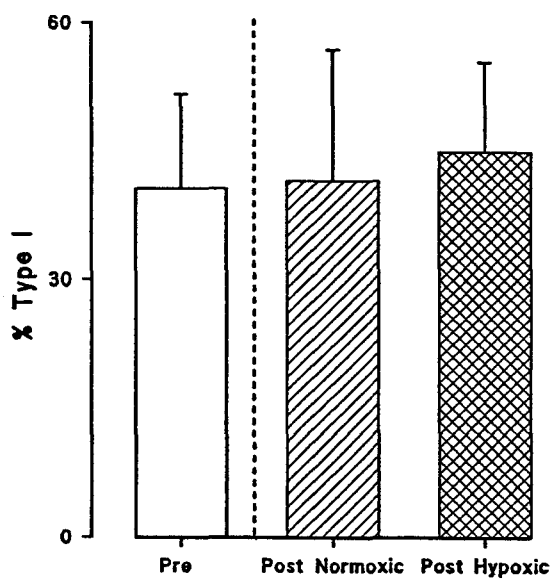
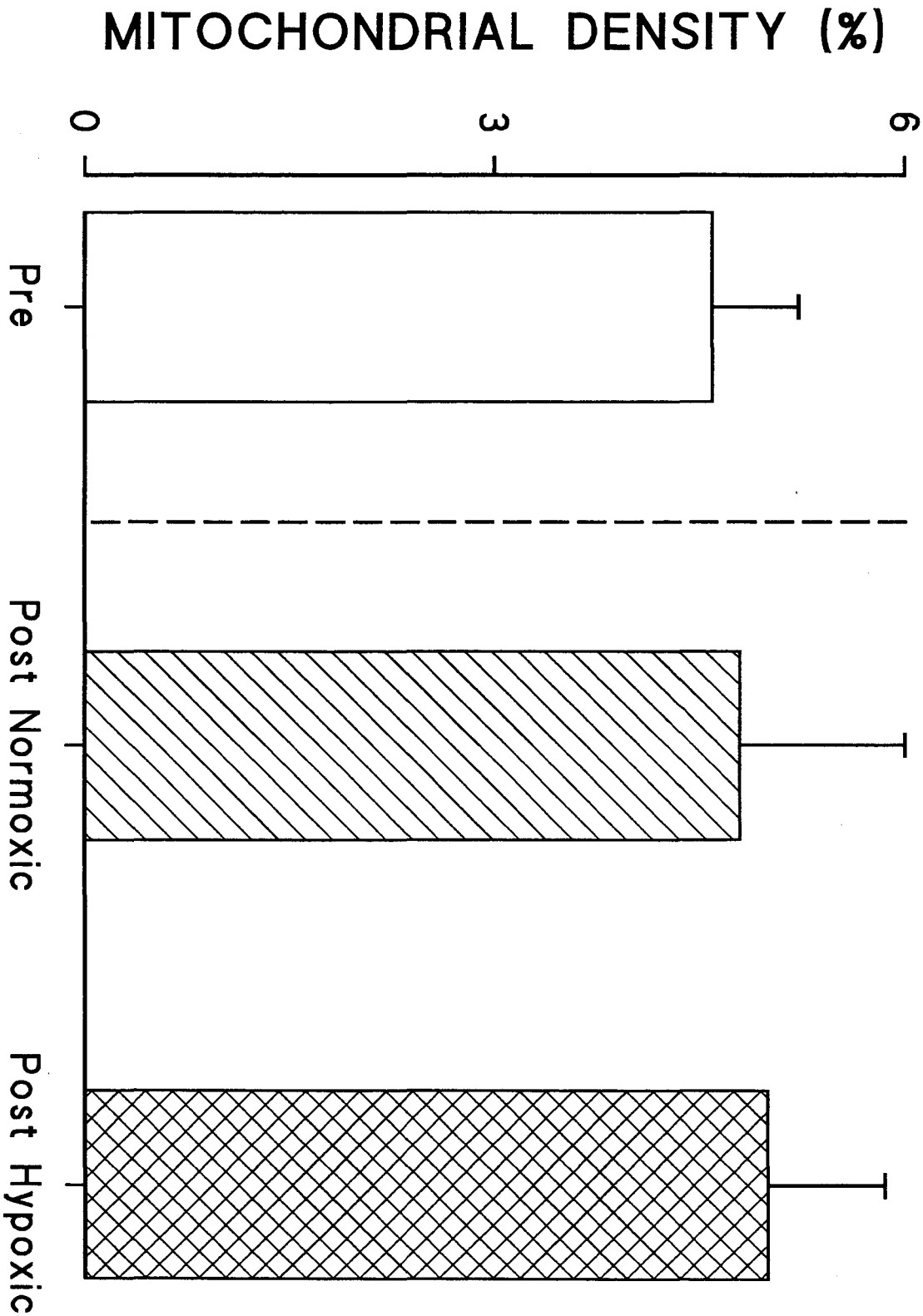
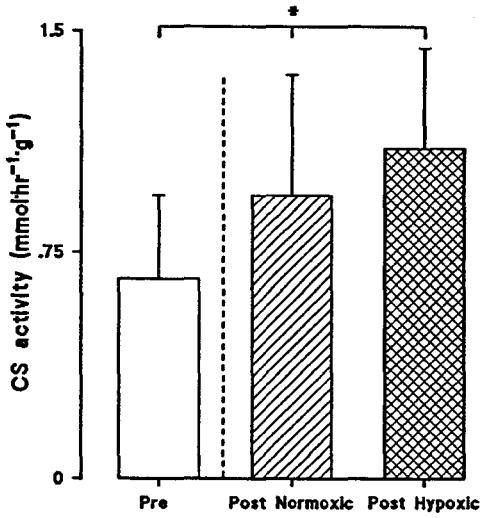
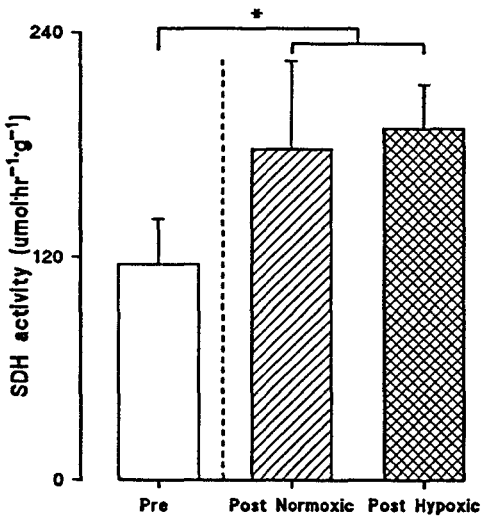

60
30
0
% Type I
Pre
Post Normoxic
Post Hypoxic

TABLE 5: Muscle ultrastructure. Data given are pre- and post-training for both the normoxically- and hypoxically-trained legs. Data includes volume density of myofibrils (V_{vmyof}), mitochondria (V_{vmit}), lipid (V_{vlip}), and cytoplasm (V_{vcyt}). Values are means ± (SD).

	PRE TRAINING	POST NORMOXIA	POST HYPOXIA
V_{vmyof} %	76.9 (4.1)	78.6 (5.0)	76.3 (2.7)
V_{vmit} %	4.6 (0.6)	4.8 (1.2)	5.0 (0.9)
V_{vlip} %	0.8 (0.4)	0.6 (0.5)	0.8 (0.4)
V_{vcyt} %	17.7 (3.8)	16.0 (4.6)	17.7 (2.8)


MITOCHONDRIAL DENSITY (%)
0
3
6
Pre
Post Normoxic
Post Hypoxic


*
1.5
.75
0
CS activity (mmol·hr⁻¹·g⁻¹)
Pre
Post Normoxic
Post Hypoxic


*
240
120
0
SDH activity (umol·hr⁻¹·g⁻¹)
Pre
Post Normoxic
Post Hypoxic

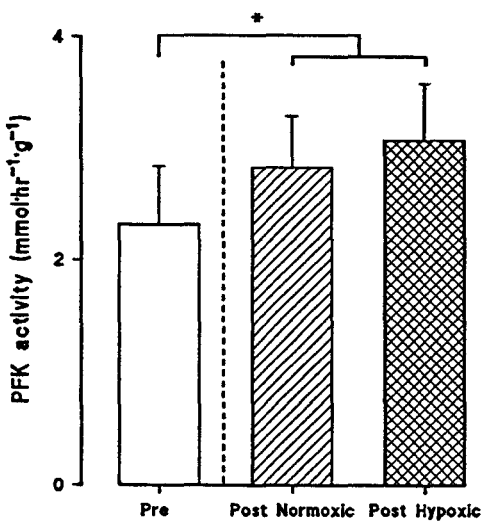

*
4
2
0
PFK activity (mmol·hr⁻¹·g⁻¹)
Pre
Post Normoxic
Post Hypoxic

TABLE 6: Enzyme activities. Data given are pre- and post-training for both the normoxically- and hypoxically-trained legs. Values are means ± (SD).

	PRE TRAINING	POST NORMOXIA	POST HYPOXIA
CS ($mmol \cdot hr^{-1} \cdot g^{-1}$)	0.66 (0.28)	0.94* (0.41)	1.10*** (0.34)
SDH ($umol \cdot hr^{-1} \cdot g^{-1}$)	115.84 (24.62)	177.95* (46.69)	188.51* (23.40)
PFK ($mmol \cdot hr^{-1} \cdot g^{-1}$)	2.31 (0.52)	2.82* (0.46)	3.06* (0.51)

* indicates $p < 0.05$; main effect for training
*** indicates $p < 0.05$; interaction

2.4 DISCUSSION

The present research involved unilateral cycling in which both legs trained at the same absolute intensity. Since absolute $\dot{V}O_2$ was the same under both conditions it was assumed that oxygen turnover and substrate flux was the same for both exercising legs and consequently, any differences found between training conditions could be attributed to adaptations at the muscle level, since the central circulatory influences were equal.

In the previously cited pilot study it was found that performance decreased when breathing an hypoxic mixture of 14 and 12.5% O_2 and the resultant $\dot{V}O_2max$ was 77 and 72% of normoxic conditions, respectively. Therefore, it should be noted that although both legs performed the same absolute amount of work, the hypoxically-trained leg was actually exercising at a higher relative intensity as compared to the normoxically-trained leg.

$\dot{V}O_2max$ improved 13 and 10% in the normoxically- and hypoxically-trained legs, respectively. Correspondingly, greater maximal power outputs were achieved as the result of higher $\dot{V}O_2max$ levels following training. Post-training values for peak blood lactate also increased 15% in the normoxically- and 17% in the hypoxically-trained legs, while

maximum HR and $\dot{V}e$ were not different for either condition pre- or post-training.

There was a very large increase in maximal aerobic capacity in both the hypoxically-trained (513%) and the normoxically-trained (402%) legs. Increases in post-training CS activity levels were significantly greater in the hypoxically-trained leg (67%) compared to the normoxically-trained leg (42%). PFK and SDH demonstrated training effects since both legs showed higher activity levels post-training. No significant differences were found in morphometry in either condition pre- or post-training.

2.4.1 Performance Measures.

2.4.1.1 Maximal Oxygen Uptake

The finding that $\dot{V}O_2max$ increased to the same extent for each leg supports previous studies which have failed to find moderate altitude training to be superior to sea level training for enhancing maximal aerobic power (Adams et al, 1975; Maher et al, 1974; Saltin, 1968; Terrados et al, 1988). Studies which infer that $\dot{V}O_2max$ can be more effectively improved by training at altitude (Buskirk et al, 1967; Mizuno et al, 1990) are often confounded by the possibility that subjects may have been relatively untrained

to begin with and that the same improvement found with altitude training would also have occurred with the equivalent amount of sea level training. In the present study, the initial level of training was not a factor as the subjects were their own controls and the two legs had equivalent levels of fitness. Thus, resultant differences in performance would be due to the training condition. Since both legs improved, and there was no difference between conditions, neither training condition was superior in enhancing $\dot{V}O_2max$.

Improvements in $\dot{V}O_2max$ can result from increases in oxygen transport from the atmosphere to the alveoli, diffusion of oxygen from the alveoli to the capillaries, cardiac output, and the diffusion of oxygen from the blood to the muscle cells (Sutton et al, 1988). Brodal et al (1977) stated that physical training increases the capacity to transport oxygen 15-30%, half of which is due to increased $\dot{Q}c$ and the other half by increased oxygen extraction (a-vO_2 diff) but more recent evidence (Åstrand et al, 1986) indicates maximum $\dot{Q}c$ to be the more dominant parameter. Although cardiac output was not measured in the present study, the finding that post-training HR during submaximal exercise at the same absolute $\dot{V}O_2$ decreased significantly (9.0%) in both legs indicates an increase in

SV and presumably maximum $\dot{Q}c$ (Rowell et al, 1986; Saltin et al, 1968).

Small increases in sea level $\dot{V}O_2max$ after altitude training can be partly explained by increases in Hgb concentration (Klausen et al, 1970). Unpublished observations found Hgb levels to increase with training, however there were no differences between conditions, pre- or post-training (Unpublished observations, undergraduate research project). Thus the increased $\dot{V}O_2$ max following training may also be partially attributed to increased Hgb concentration, but in the present study this would have no effect on between condition comparisons.

Maximal post-training HR was not different for either leg. This demonstrated that the subjects actually performed maximal exercise pre- and post-training. Since maximal HR was unaffected by training, or condition, it may be concluded that the increase in $\dot{V}O_2max$ after training was a function of either an increase in flow (SV and resultant $\dot{Q}c$) or a larger a-vO_2 difference. Saltin (1968) demonstrated a decrease of 15% in maximal $\dot{Q}c$ primarily due to a smaller SV and, to a lesser extent, a lower HR following prolonged altitude exposure. Pugh (1964) concluded that a reduced plasma volume at altitude contributed to a lower maximal SV with a reduced HR to

reduce $\dot{Q}c_{max}$, while others have explained the lower HR to reflect a greater arteriovenous oxygen difference from decreased Hgb-oxygen affinity (Faulkner et al, 1967; Mairbaurl et al, 1986). The fact that HR_{max} did not change in the present research, but $\dot{V}O_2max$ improved, demonstrates that $\dot{Q}c_{max}$ was not reduced.

Peak blood lactate concentrations were higher post-training. Increases of 15.0 and 16.8% were found in the normoxically- and hypoxically-trained legs, respectively. Mizuno et al (1990) found acclimatized individuals performing at sea level to have a 6% increase in muscle buffer capacity, and he concluded that hypoxia was the critical factor since it has been shown that endurance training does not seem to be of significance in muscle buffer capacity changes (Parkhouse et al, 1985; Sahlin et al, 1984). The higher peak blood lactate levels in the present study, however, were not caused by the hypoxic factor since there was no difference between legs (Faulkner et al, 1967). Mizuno et al (1990) also noted that the increased buffer capacity allowed a greater anaerobic energy yield, which would agree with our results of higher blood lactate levels and increased anaerobic enzyme activities.

The higher peak blood lactate concentrations suggests a higher tolerance for [H+] during exhausting work

(Faulkner et al, 1967). It is probable that the high intensity (100% pre-training maximal power output) interval training resulted in higher anaerobic metabolism and greater peak blood lactate levels in both legs post-training. It could be suggested that the higher power output reached following training required a larger portion of type II glycolytic fibers to be recruited resulting in more anaerobic metabolism and resultant lactate formation (Holloszy, 1973; Shephard et al, 1988). Since higher power outputs were reached, the length of the test was longer, allowing more time for the lactate to diffuse from the active muscles into the blood (Shephard et al, 1988).

Many have found reduced maximal blood lactate levels following chronic altitude exposure (Bender et al, 1989; Brooks et al, 1991; Cerretelli et al, 1982; Edwards, 1936; Green et al, 1989; Maher et al, 1974; West, 1986). This has been explained as a reduced buffering capacity causing decreases in intracellular pH to "turn off" enzymes in the glycolytic pathway (Cerretelli et al, 1982, 1990). Lower anaerobic glycolytic flux rate may be due to reductions in activation of the contractile apparatus as a protective mechanism from metabolic acidosis (Green et al, 1989). In the present study, subjects were only exposed to hypoxia during their training sessions and thus they avoided many of

the maladaptations which may occur with chronic altitude exposure.

Any central circulatory effects of training can be expected to affect the performance of both legs in a similar way and cannot explain differences attained between the two legs (Sundberg et al, 1993). Because the legs were trained at the same intensity, the oxygen turnover and the substrate flux were probably of the same magnitude (Terrados et al, 1990). Any training influences to circulatory changes which enhance $\dot{V}O_2max$ would be equal in both legs. Ventilation and blood flow can not be restricted to one side of the body and therefore the effects improve both legs regardless of training condition.

2.4.1.2 Maximal Aerobic Capacity

Large improvements in MAC were seen in both the normoxically- (402%) and hypoxically- (513%) trained legs. Mairbaurl (1986) and others (Maher et al, 1974; Terrados et al, 1990) showed an increase in MAC upon return to sea level after training at moderate altitude, while others did not find improvements in work to exhaustion after 2 weeks at 4,300 meters (Hansen et al, 1967).

Local aerobic capacity of human skeletal muscle improves in response to endurance training through increases

in oxidative enzyme activity and capillary density (Saltin et al, 1983). Sundberg et al (1993) found a reduction in oxygen supply to be an additive stimulus for the adaptation to endurance training. In the present study, MAC increased five fold for the hypoxically-trained leg and four fold for the normoxically-trained leg but this difference was not significant between legs. This was surprising in light of the enhanced CS activity which was found in the muscle of the hypoxically-trained legs and may have been due to the protocol which was used to assess MAC.

The increases in MAC which were found following training were considerably greater than expected. Although part of this is obviously due to the fact that the same absolute load represented a lower relative intensity for the normoxically-trained leg, part of this may also have been due to a "learning effect". Following training, subjects may have improved the mechanical efficiency for unilateral cycling. Oxygen consumption at the same point in time during the MAC test was significantly ($p < 0.05$) lower in both the normoxically- (18%) and hypoxically- (25%) trained legs. Therefore, subjects were able to perform the same power output with lower $\dot{V}O_2$ following 8 weeks of unique unilateral cycling. It could also be suggested that post-training MAC was not to a point of "fatigue" since many

subjects quit due to pain in their backs, dehydration, and "boredom".

2.4.2 Physiological Measures at the Same Absolute Submaximal Power Output

As expected oxygen uptake at the same submaximal power output did not change following training but HR and $\dot{V}e$ were lower for both legs. Because there were no differences between legs, this represents a training effect and not an influence of hypoxia. The fall in HR is probably related to an increase in parasympathetic nervous system activity or an increase in SV following training (Sime et al, 1971).

Much research (Hermansen et al, 1971; Klausen et al, 1969) has shown decreased submaximal blood lactate levels following acclimatization (reduced PaO_2). The present study, however, found no difference in training condition pre- or post-training. Therefore the level was independent of PIO_2 or training.

The reduced $\dot{V}e$ is interpreted as being due to a lower relative exercise intensity following training caused by the increase in $\dot{V}O_2max$. The new relative intensity is thus further removed from the ventilatory threshold than in the pre-training condition.

2.4.3 Biochemical Data

Holloszy (1973) demonstrated that increases in mitochondrial enzyme activities are regularly induced by submaximal endurance training. The present study demonstrated increases due to training in both CS and SDH activity levels, probably because the subjects were not endurance trained athletes previous to the training program and their skeletal muscle oxidative capacity was not already high (Terrados et al, 1988). Although one might normally expect changes in one oxidative enzyme to parallel changes in another, changes in CS activity have previously been noted independent of other oxidative enzymes (Sale et al., 1990; Green et al., 1989). Thus the greater increase in the oxidative capacity (as marked by the more sensitive enzyme CS) of the hypoxically-trained muscle may reflect a preferential change in the mitochondrial fraction of the cell indicating that certain respiratory enzymes may be involved in acclimatization to moderate altitude (Andersen et al, 1977; Reynafarje, 1962; Terblanche et al, 1983; Terrados et al, 1990). Extreme hypoxia on the other hand is responsible for a decline in oxidative potential (Green et al, 1989; Howald et al, 1990), while others have found no change in oxidative enzyme activities following training and altitude exposure (Young et al, 1982).

Contrary to previous findings (Green et al, 1989; Young et al, 1982), glycolytic enzyme activity also increased post-training. Since the PFK activity was not different between trained legs, the glycolytic enzymes were not significantly involved in the adaptive process to hypoxia (Reynafarje, 1962). Increased PFK activity may have been due to the interval training in which power outputs equivalent to 100% pre-training maximal power outputs were maintained. Others have shown that high work intensities (at or above $\dot{V}O_2max$) are a prerequisite for a training effect on glycolytic capacity (Howald et al, 1990; Terrados et al, 1988), with others support the fact that glycolytic enzyme activities undergo small modifications with training, depending on the intensity and type of training (Gollnick et al, 1973).

2.4.4 Morphometric Data

2.4.4.1 Capillary/Fiber Ratio and Capillary Density

The mean number of capillaries per fiber and capillary density were 15.6% and 5.3% higher in the normoxically-trained leg and 26.2% and 9.8% higher in the hypoxically-trained leg, respectively, but these differences were not statistically significant. Thus one must conclude that little or no changes occurred in the diffusion distance

for oxygen or the distribution of blood flow within the working muscle (Mizuno et al, 1990; Sundberg, et al, 1990). Evidence has shown the capillary per fiber ratio to be 1.0-1.8 in untrained and up to 2.5 in trained individuals, while the capillary densities per mm^2 may be 305 and 425, respectively (Brodal et al, 1977; Hermansen et al, 1971; MacDougall et al, 1991).

Capillary density in the present research was low both pre- and post-training (123-135 per mm^2); however, the subjects had never performed endurance training and therefore were more representative of untrained individuals. Following 40 days of extreme hypoxia MacDougall et al (1991) found no change in capillary to fiber ratio with a tendency for higher capillary density. It is known that training may lead to increases in the number of capillaries around each fiber (Andersen et al, 1975, 1977; Brodal et al, 1977) as well as an increase in fiber diameter (hypertrophy). Because of this, if the hypertrophy is more pronounced, the capillary density (per mm^2) may not increase. Physical exercise influences the capillary/fiber ratio but capillary density depends on the hypertrophy induced (Brodal et al, 1977). With a greater fiber diameter, the capillaries are spread farther apart and the density decreases while the ratio may remain constant (Hudlicka, 1982). Since the

present results did not show any significant differences in fiber cross-sectional area this mechanism would not have influenced capillary density.

Fiber growth can rearrange the capillary network, as increases may be mediated either by an increase in capillary number or by reduction in fiber size. Increases in the number of capillaries are effective in shortening the diffusion distance when the capillary density is low, but are much less effective when the capillary density is already high (Banchero, 1987). When sedentary individuals follow an endurance program, the increase in capillary supply is larger than the increase in cross-sectional area, resulting in a shorter diffusion distance, however, in endurance trained athletes, a larger number of capillaries and a reduced muscle fiber area can be seen for an even shorter diffusion distance (Hermansen et al, 1971; Saltin et al, 1983).

It has been shown that fibers containing many mitochondria (greater oxygen extraction capacities) are surrounded by more capillaries than fibers with few mitochondria (Brodal et al, 1977; Hoppeler et al, 1981). Highly oxidative muscle fibers have a higher capillary density and are usually smaller, therefore the capillary per fiber ratio is influenced by larger differences than capillary density compared to the values for larger

glycolytic fibers (Hudlicka, 1982). Our results support this finding since neither mitochondrial density or capillary density changed as a result of training.

It should be noted that several studies have demonstrated that the identification of capillaries may be difficult with the use of a light microscope (Andersen, 1975; Brodal et al, 1977; Hermansen et al, 1971; Plyley et al, 1975). The fact that not all capillaries are packed with red blood cells nor with perfusion medium further complicates the visualization of such structures (Plyley et al, 1975). Hermansen et al (1971) demonstrated that there was no difference between capillary density (per mm^2) values at rest or during exercise with the use of periodic-acid-Schiff staining. This technique allows the capillaries to be counted regardless of whether or not they are filled with erythrocytes, or are open or closed. Even with the aid of staining techniques, there is a resultant wide range of values for capillary density (Brodal et al, 1977). Others have suggested that there is a shrinkage or swelling of tissues during the histochemical procedures which affects results (Brodal et al, 1977); however, the capillary per fiber ratio would not be affected by this response. In addition since pre- and post-training tissue was prepared

identically and analyzed on the same day, such changes should not have affected between condition comparisons.

2.4.4.2 Fiber Area and Fiber Type Distribution

There were no significant difference in fiber CSA for either condition following training. Increases in type II fiber area has been previously shown by Mizuno et al (1990). This is not surprising since many training studies (Holloszy, 1973) have shown type II fibers to be influenced by hypertrophy as compared to type I fibers. The CSA of the fibers in the present study did not change and therefore there was not an overall loss in muscle tissue as has been seen under severe chronic hypoxia (Hoppeler et al, 1990). Extreme hypoxia leads to a reduction in muscle mass and comparable decreases in both type I and type II fiber areas (Green et al, 1989; MacDougall et al, 1991). As expected, there was no change in the percentage of type I and II fibers in either leg, pre- or post-training although there may have been changes in fiber sub-types.

2.4.4.3. Muscle Ultrastructure

Under severe hypoxia, muscle tissue loss indirectly reduces mitochondrial volume density (Hoppeler, 1990; Howald et al, 1990). It has been shown that the oxidative capacity of a tissue is proportional to the number and volume of

mitochondria. However, Ferretti et al (1990) found that although there was a reduction in muscle mass and CSA, there was not a loss in muscle function as seen by maintained muscular power levels. Since the CSA of the muscle tissue, in the present study did not decrease, there was not an accompanied reduction in absolute mitochondrial volume. MacDougall et al (1991) observed a trend towards higher mitochondrial volume density and concluded that this would result in decreased diffusion distance for oxygen from the cell membrane to the mitochondria. These researchers also explained that the mitochondrial to myofibrillar volume ratio only tended to increase while the decreased fiber area indicated that the number of mitochondria did not increase but may have decreased. The resulant mitochondrial volume density of this study (4.6-5.0%) is similar to that found by others (Hoppeler et al, 1990; MacDougall et al, 1991). The fact that mitochondrial volume density did not show a proportionate increase with CS activity was a surprising and unexpected finding since the two are usually tightly coupled (Hoppeler, 1990). It may be that qualitative changes in the mitochondrial matrix occurred which were independent of quantitative measurements of mitochondrial volume.

2.5 SUMMARY AND RECOMMENDATIONS

Training resulted in higher $\dot{V}O_2max$, and maximal power output, $\dot{V}e$ and blood lactate concentrations, but there were no differences between training conditions. Hypoxia combined with exercise training significantly increased CS activity over and above that which occurred with the same training under normoxic conditions, but these changes were not reflected in similar increases in MAC. These findings may be interpreted in at least 3 different ways:

1. Based on the changes which were found in the oxidative marker CS, one might conclude that the combination of moderate hypoxia and training enhances adaptations at the muscle level to a greater extent than that which occurs under normoxic conditions. The fact that these biochemical changes did not translate directly into a greater exercise performance with the hypoxically-trained leg may relate to the method which was used to quantify aerobic capacity.
2. Based on the measurement of MAC, one might conclude that these biochemical changes are not important to performance and that the

combination of hypoxia and training is no more effective than performing the same amount of training under normoxic conditions.

3. The training protocol which was utilized was not of sufficient intensity, volume and/or duration to clearly discriminate between the 2 conditions. Thus, while it may have been effective in stimulating some of the more sensitive adaptations at the muscle level, the magnitude of other adpatations (e.g., morphometric or performance changes) was not sufficient to be detected by the measurements which were used.

Further investigation of this topic is necessary before definitive conclusions can be reached.

A training protocol where subjects only experience hypoxia while they are training (as in the present study) may be superior to actual training at altitude. Although a sojourn at altitude may result in enhanced oxygen carrying capacity due to elevated Hgb such adaptations may be off-set by maladaptations such as reduced maximal $\dot{Q}c$ (MacDougall et al, 1976; Saltin et al, 1967), muscle atrophy (Green et al, 1989; Hoppeler et al, 1990; Howald et al, 1990; MacDougall

et al, 1991) or reduced buffer capacity and anaerobic power (Bender et al, 1989; Brooks et al, 1991; Cerretelli et al, 1982; Edwards, 1936; Green et al, 1989; Maher et al, 1974; West, 1986). With a protocol as in the present study the hypoxic exposure time is so brief that these negative effects are probably avoided. The method used to simulate altitude is inexpensive and adaptable to a number of sports, and does not necessitate transportation of athletes to altitude for training.

2.6 REFERENCES

Adams, W.C., Bernauer, E.M., Dill, D.B., and Bomar, J.B., Jr. (1975) Effects of equivalent sea level and altitude training on VO_2max and running performance. J. Appl. Physiol. 39(2): 262-268.

Alexander, J.K., Hartley, L.H., Modelski, M., and Grover, R.F. (1967) Reduction of stroke volume during exercise in man following ascent to 3,100 m altitude. J. Appl. Physiol. 23(6): 849-858.

Andersen, P. (1975) Capillary density in skeletal muscle of man. Acta. Physiol. Scand. 95: 203-205.

Andersen, P., and Hendriksson, J. (1977) Capillary supply of the quadriceps femoris muscle of man: adaptive response to exercise. J. Physiol. (London) 270: 677-690.

Anderson, H.T., Smeland, E.B., Owe, J.O., and Myhre, K. (1985) Analyses of maximal cardiopulmonary performance during exposure to acute hypoxia at simulated altitude-sea level to 5,000 meters. Aviat. Space Environ. Med. 56: 1192-1197.

Åstrand, P.O., and Rodahl, K. (1986) Textbook of Work Physiology. 3rd. Ed. New York, N.Y.: McGraw-Hill.

Balke, B. (1964) Work capactiy and it's limiting factors at high altitude. In Physiological Effects of High Altitude. New York, N.Y.: Macmillan, pp. 233-247.

Balke, B., Nagle, F.J., and Daniels, J.T. (1965) Altitude and maximum performance in work and sports activity. JAMA. 194: 176-179.

Banchero, N. (1975) Capillary density of skeletal muscle in dogs exposed to simulated altitude. Proc. Soc. Exp. Biol. Med. 148: 435-439.

Banchero, N. (1987) Cardiovascular responses to chronic hypoxia. Ann. Rev. Physiol. 49: 465-476.

Bebout, D.E., Story, D., Roca, J., Hogan, M.C., Poole, D.C., Gonzalez-Camarena, R., Ueno, O., Haab, P., and Wagner, P.D. (1989) Effects of altitude acclimatization on pulmonary gas exchange during exercise. J. Appl. Physiol. 67(6): 2286-2295.

Bender, P.R., Bertron, M.G., McCullough, R.G., Trad, L., Young, A.J., Cymerman, A., and Reeves, J.T. (1989) Decreased exercise muscle lactate release after high altitude acclimatization. J. Appl. Physiol. 67(4): 1456-1462.

Berglund, B. (1992) High-altitude training: aspects of haematological adaptation. Sports Med. 14(5): 289-303.

Bergström, C.J. (1962) Muscle electrolytes in man. Scand. J. Clin. Lab Invest. Suppl 68: 1-110.

Bigard, A., Brunet, A., Guezennec, C.Y., and Monad, H. (1991) Skeletal muscle changes after endurance training at higher altitude. J. Appl. Physiol. 71: 2114-2121.

Boutellier, U., Howald, H., DiPrampero, P.E., Giezendanner, D., and Cerretelli, P. (1983) Human muscle adaptations to chronic hypoxia. Proc. 3rd. Banff Int. Hypoxia Symp. pp. 273-281.

Boutellier, U., Giezendanner, D., Cerretelli, P., and DiPrampero, P.E. (1984) After effects of chronic hypoxia on VO_2max kinetics and O_2 deficit and debt. Eur. J. Appl. Physiol. Occup. Physiol. 53: 87-91.

Brodal, P., Ingjer, F., and Hermansen, L. (1977) Capillary supply of skeletal muscle fibres in untrained and endurance trained men. Am. J. Physiol. 232(6): H705-712.

Brooks, G.A., and Fahey, T.D. (1985) Eds. Exercise Physiology: Human Bioenergetics and it's Applications. New York, N.Y.: Macmillan Publishing Company.

Brooks, G.A., Butterfield, G.E., Wolfe, R.R., Broves, B.M., Mazzeo, R.S., Sutton, J.R., Wolfel, E.E., and Reeves, J.T. (1991) Decreased reliance on lactate during exercise after acclimatization to 4,300 m. J. Appl. Physiol. 71(1): 333-341.

Buskirk, E.R. (1966) Physiology and performance of track athletes at various altitudes in the United States and Peru. In The Effects of Altitude on Physical Performance. P.F. Goddard, Ed. Chicago, IL: Athletic Inst., pp.65-72.

Buskirk, E.R., Kollias, J., Akers, R.E., Prokop, E.K. and Reategui, E.P. (1967) Maximal performance at altitude and return from altitude in conditioned runners. J. Appl. Physiol. 23: 259-266.

Cassin, S., Gilbert, R.D., Bunnell, C.E., and Johnson, E.M. (1971) Capillary development during exposure to chronic hypoxia. Am. J. Physiol. 220(2): 448-451.

Cerretelli, P. (1976) Limiting factors to oxygen transport on Mount Everest. J. Appl. Physiol. 40: 658-667.

Cerretelli, P. (1980) Gas Exchange at high altitude. In West. I. Ed: Pulmonary gas exchange Vol. 2 Organism and Environment. New York, N.Y.: Academic Press, pp. 97-147.

Cerretelli, P., Veicsteinas, A., and Marconi, C. (1982) Anaerobic metabolism at high altitude: the lactacid mechanism. In High Altitude Physiology and Medicine. W. Brendel and R.A. Zink Eds. New York, N.Y.: Spronger Verlag, pp.94-102.

Cerretelli, P. and Binzoni, T. (1990) The energetic significance of lactate accumulation in blood at altitude. J. Sports Med. 11 (Suppl 1): S27-S30.

Daniels, J.T., and Oldridge, N. (1970) The effects of alternate exposure to altitude and sea level on world class middle distance runners. Med. Sci. Sports 2: 107-112.

Dill, D.B., and Adams, W.C. (1971) Maximal oxygen uptake at sea level and at 3,090 m altitude in high school champion runners. J. Appl. Physiol. 30: 854-859.

Dill, D.B., Braithwaite, K., Adams, W.C., and Bernauer, E.M. (1974) Blood volume of middle-distance runners: effect of 2,300 m altitude and comparison with nonathletes. Med. Sci. Sports 6: 1-7.

Edwards, H.T. (1936) Lactic acid in rest and work at high altitude. Am. J. Physiol. 116: 367-375.

Escourrou, P., Johnson, D.G., and Rowell, L.B. (1984) Hypoxemia increases in plasma catecholamine concentrations in exercising humans. J. Appl. Physiol. 57(5): 1507-1511.

Essen, B., Jansson, E., Henriksson, J., Taylor, A.W., and Saltin, B. (1975) Metabolic characteristics of fibre types in human skeletal muscle. Acta. Physiol. Scand. 95: 153-165.

Faulkner, J.A., Daniels, J.T., and Balke, B. (1967) Effects of training at moderate altitude on physical performance capacity. J. Appl. Physiol. 23(1): 85-89.

Faulkner, J.A., Kollias, A.J., Favour, C.B., Buskirk, E.R., and Balke, B. (1968) Maximal aerobic capacity and running performance at altitude. J. Appl. Physiol. 24: 685-691.

Ferretti, G., Boutellier, U., Pendergast, D.R., Moia, C., Minetti, A.E., Howald, H., and DiPrampero, P.E. (1990) Oxygen transport system before and after exposure to chronic hypoxia. Int. J. Sports Med. 11 (Suppl 1): S15-S20.

Fox, E.L., Bowers, R.W., and Foss, M.L. (1988) Eds. The Physiological Basis of Physical Education and Athletics. 4th. Ed. Dubuque, IA: Wm. C. Brown Publishers.

Gollnick, P.D., Armstrong, R.B., Saltin, B., Saubert, C.B., Sembrowhich, W.L., and Shepherd, R.E. (1973) Effects of training on enzymes activities and fibre composition of human skeletal muscle. J. Appl. Physiol. 34: 107-111.

Green, H.J., Sutton, J.R., Young, P.M., Cymerman, A., and Houston, C.S. (1989) Operation Everest II: adaptations in human skeletal muscle. J. Appl. Physiol. 66(5): 2454-2461.

Grover, R.F., Reeves, J.T., Grover, E.B., and Leathers, J.E. (1967) Muscular exercise in young men native to 3,100 m altitude. J. Appl. Physiol. 22: 555-564.

Grover, R.F., Lufschanowsky, R., and Alexander, J.K. (1976) Alterations in the coronary circulation of man following ascent to 3,100 m altitude. J. Appl. Physiol. 41: 832-838.

Hansen, J.E., Vogel, J.A., Stelter, G.P., and Consolazio, C.F. (1967) Oxygen uptake in man during exhaustive work at sea level and high altitude. J. Appl. Physiol. 23(4): 511-522.

Hansen, J.E., Stelter, G.P., and Vogel, J.A. (1975) Arterial pyruvate, lactate, pH, and PCO_2 during work at sea level and high altitude. J. Appl. Physiol. 23: 523-530.

Hartley, L.H., Alexander, J.K., Modelski, M., and Grover, R.F. (1967) Subnormal cardiac output at rest and during exercise in residents at 3,100 m altitude. J. Appl. Physiol. 23(6): 839-848.

Hartley, L.H., Vogel, J.A., and Landown, M. (1973) Central, femoral, and brachial circulation during exercise in hypoxia. J. Appl. Physiol. 34: 87-90.

Hermansen, L., and Wachtlova, M. (1971) Capillary density of skeletal muscle in well trained and untrained men. J. Appl. Physiol. 30: 860-863.

Holloszy, J.O. (1973) Biochemical adaptations to exercise aerobic metabolism. Exerc. Sport Sci. Rev. 1: 45-71.

Hoppeler, H., Luthi, P., Claassen, H., Weibel, E.R., and Howald, H. (1973) The ultrastructure of the normal human skeletal muscle. A morphometric analysis on untrained men, women, and well-trained orienteers. Pflugers Arch. 344: 217-232.

Hoppeler, H., Mathieu, O., Weibel, E.R., Krauer, R., Lindstedt, S.L., Taylor, C.R. (1981) Design of the mammalian respiratory system. Capillaries in skeletal muscle. Respir. Physiol. 44: 129-156.

Hoppeler, H., Kleinert, E., Schlegel, C., Claassen, H., Howald, H., Kayar, S.R., and Cerretelli, P. (1990) Morphological adaptations of human skeletal muscle to chronic hypoxia. Int. J. Sports Med. 11(Suppl 1): S3-S9.

Howald, H., Pette, D., Simoneau, J.A., Uber, A., Hoppeler, H., and Cerretelli, P. (1990) Effects of chronic hypoxia on muscle enzyme activities. Int. J. Sports Med. 11(Suppl 1): S10-S14.

Hudlicka, O. (1982) Growth of capillaries in skeletal and cardiac muscle. Circ. Research 50(4): 451-461.

Jones, N.L., Robertson, D.G., Kane, J.W., and Hart, A.W. (1972) Effect of hypoxia on free fatty acid metabolism during exercise. J. Appl. Physiol. 33(6): 733-738.

Klausen, K. (1966) Cardiac output in man in rest and work during and after acclimatization to 3,800 meters. J. Appl. Physiol. 21: 609-616.

Klausen, K., and Forster, H. (1969) The effect of acute reduction in air density on minute ventilation during exercise. Acta. Physiol. Scand. Suppl. 330: 81.

Klausen, K., Dill, D.B., and Horvath, S.M. (1970) Exercise at ambient and high oxygen pressure at high altitude and at sea level. J. Appl.Physiol. 29(4): 456-463.

Levine, B.D., and Stray-Gundersen, J. Altitude training does not improve running performance more than equivalent training near sea level in trained runners. Med. Sci. Sports Exerc. in press.

Lewis, S.M. (1989) Erythropoiesis. In Postgraduate haematology, Heineman Professional Publishing.

Lowry, O.H., and Passonneau, J.V. (1972) A flexible system of enzymatic analysis 1st ed. New York, N.Y.: Academic.

MacDougall, J.D., Reddan, W.G., Dempsey, J.A., and Forster, H. (1976) Acute alterations in stroke volume during exercise at 3,100 m altitude. J. Human Ergol. 5: 103-111.

MacDougall, J.D., Green, H.J., Sutton, J.R., Coates, G., Cymerman, A., Young, P., and Houston, C.S. (1991) Operation Everest II: structural adaptations in skeletal muscle in response to extreme simulated altitude. Acta. Physiol. Scand. 142: 421-427.

Maher, J.Y., Jones, L.G., and Hartley, L.H. (1974) Effects of high altitude exposure on submaximal endurance capacity of men. J. Appl. Physiol. 37: 895-898.

Mairburl, H., Schibersberger, W., Humpeler, F., Hasibeder, W., Fischer, W., and Raas, E. (1986) Beneficial effects of exercising at moderate altitude on red cell oxygen transport and on exercise performance. Pluegers Arch. 406: 594-599.

Manchanda, S.C., Maher, J.T., and Cymerman, A. (1975) Cardiac performance during graded exercise in acute hypoxia. J. Appl. Physiol. 38(5): 858-862.

McManus, B.M., Horvath, S.M., Bolduan, N., and Miller, J.C. (1974) Metabolic and cardiorespiratory responses to long term work under hypoxic conditions. J. Appl. Physiol. 36(2): 177-182.

Mizuno, M., Secher, N.H., and Saltin, B. (1985) Fibre types, capillary supply and enzyme activities in human intercostal muscles. Clin. Physiol. Oxf. 5: 121-135.

Mizuno, M., Juel, C., Bro-Rasmussen, T., Mygind, T., Schibye, B., Rasmussen, B., and Saltin, B. (1990) Limb skeletal muscle adaptation in elite athletes after training at altitude. J. Appl. Physiol. 68: 496-502.

Moret, P., Covarrubias, E., Coudert, J., and Duchosal, F. (1972) Cardiovascular adaptation to chronic hypoxia. Acta. Cardiol. 27: 283-305.

Padykula, H.A., and Herman, E. (1955) The specificity of the histochemical method of adenosinetriphosphatase. J. Histochem. Cytochem. 3: 170-195.

Parkhouse, W.S., McKenzie, D.C., Hochachka, P.W., and Ovalle, W.K. (1985) Buffering capacity of deproteinized human vastus lateralis muscle. J. Appl. Physiol. 58: 14-17.

Plyley, M.J., and Groom, A.C. (1975) Geometrical distribution of capillaries in mammalian striated muscle. Am. J. Physiol. 228: 1376-1383.

Pugh, L.G.C.E., Gill, M.B., Lahiri, S., Milledge, J.S., Ward, M.P., and West, J.B. (1964) Muscular exercise at great altitudes. J. Appl. Physiol. 19(3): 431-440.

Reeves, J.T., Groves, B.M., Sutton, J.R., Wagner, P.D., Cymerman, A., Malconian, M.K., Rock, P.B., Young, P.M., and Houston, C.S. (1987) Operation Everest II: preservation of cardiac function at extreme altitude. J. Appl. Physiol. 63(2): 531-539.

Reynafarje, B. (1962) Myoglobin content and enzymatic activity of muscle and altitude adaptation. J. Appl. Physiol. 17(2): 301-305.

Reynafarje, C., Lorano, R., and Valdivieso, J. (1959) The polycythemia of high altitudes: iron metabolism and related aspects. Blood. 14: 433-455.

Reynafarje, C., Faura, J., and Villavicencio, D. (1975) Oxygen transport of hemoglobin in high altitude animals. J. Appl. Physiol. 38: 806-810.

Rowell, L.B., Saltin, B., Keins, B., and Christensen, J.N. (1986) Is peak quadriceps blood flow in humans even higher during exercise with hypoxemia? Am. J. Physiol. 251: H1038-1044.

Sahlin, K. and Henriksson, J. (1984) Buffer capacity and lactate accumulation in skeletal muscle of trained and untrained man. Acta. Physiol. Scand. 122: 331-339.

Saltin, B. (1968) Maximal oxygen uptake and cardiac output after two weeks at 4,300 meters. J. Appl. Physiol. 25: 400-409.

Saltin, B. (1969) Physiological effects of physical training. Med. Sci. Sports 1: 50-56.

Saltin, B., and Gollick, P.D. (1983) Skeletal muscle adaptability: significance for metabolism and performance. In Skeletal Muscle. L.E. Peachey Ed. Edthesda, M: American Physiological Society, pp. 555-631.

Sale, D.G., J.D. MacDougall, I. Jacobs and S. Garner. (1990) Interaction between concurrent strength and endurance training. J. Appl. Physiol. 68: 260-270.

Sawka, M.N., and Young, A.J. (1989) Acute polycythemia and human performance during exercise and exposure to extreme environments. In Exercise and Sport Science Reviews K.B. Pandolf Ed. 17: 265-293.

Schibye, B., Klausen, K., Trap-Jensen, J., Lund, J.O., and Hartling, O. (1988) Effects of acute hypoxia and carbon dioxide inhalation on systematic and peripheral oxygen uptake and circulatory responses during moderate exercise. Eur. J. Appl. Physiol. Occup. Physiol. 57: 519-525.

Shephard, R.J., Bouhlel, E., Vanderwalle, H., and Monod, H. (1988) Peak oxygen intake and hypoxia: influence of physical fitness. Int. J. Sports Med. 9(9): 279-283.

Sime, F., Penaloza, D., and Ruiz, L. (1971) Bradycardia, increased cardiac output, and reversal of pulmonary hypertension in altitude natives living at sea level. Brit. Heart J. 33: 647-657.

Sundberg, C.J., Eiken, O., Esbjornsson, M., Nygren, A., and Kaijser, L. (1990) Effects of training with reduced perfusion pressure on performance and muscle fibre characteristics. In Proceedings of the Fourth European Symposium on Life Sciences research in space. Trieste, Italy, pp.197-198.

Sundberg, C.J., Eiken, O., Nygren, A., and Kaijser, L. (1993) Effects of ischaemic training on local aerobic muscle performance in man. Acta. Physiol. Scand. 148: 13-19.

Sutton, J.R. (1977) The effect of acute hypoxia on the hormonal response to exercise. J. Appl. Physiol. 42: 587-592.

Sutton, J.R., Jones, N.L., and Toews, C. J., (1981) The effect of pH on muscle glycolysis during exercise. Clin. Sci. Lond. 61: 331-338.

Sutton, J.R., Reeves, J.T., Wagner, P.D., Groves, B.M., Cymerman, Y., Malconian, M.K., Rock, P.B., Young, P.M., Walter, S.D., and Houston, C.S. (1988) Operation Everest II: oxygen transport during exercise at extreme simulated altitude. J. Appl. Physiol. 64: 1309-1321.

Sutton, J.R., Coates, G., and Remmers, J.E. (1990) Hypoxia: The Adaptations. Eds. Toronto, Canada: B.C. Decker Inc.

Terblanche, S.E., Groenewald, J.V., van der Linde, A., Wolfswinkel, J.M., Jooste, P.L. and Oelofsen, W. (1983) A comparative study on the effect of training at altitude and at sea level on endurance and certain biochemical variables. Comp. Biochem. Physiol. 78A(1): 21-26.

Terrados, N., Mizuno, M., and Andersen, H. (1985) Reduction in maximal oxygen uptake at low altitudes; role of training status and lung function. Clin. Physiol. 5(Suppl 3): 75-79.

Terrados, N., Melichna, J., Sylven, C., Jansson, E., and Kaijser, L. (1988) Effects of training at simulated altitude on performance and muscle metabolic capacity in competitive road cyclists. Eur. J. Appl. Physiol. Occup. Physiol. 57: 203-209.

Terrados, N., Jansson, E., Sylven, C., and Kaijser, L. (1990) Is hypoxia a stimulus for synthesis of oxidative enzymes and myoglobin? J. Appl. Physiol. 68(6): 2369-2372.

Valdivia, E. (1958) Total capillary bed in striated muscle of guinea pigs native to the Peruvian mountains. Am. J. Physiol. 194: 585-589.

Wagner, J.A., Miles, D.S., and Horvath, S.M. (1980) Physiological adjustments of women to prolonged work during acute hypoxia. J. Appl. Physiol. 49(3): 367-373.

Weibel, E.R. (1979) Stereological Methods. Vol I: Pratical Methods for Biological Morphometry. Toronto: Academic Press, Chapters 4 & 6.

West, J.B. (1983) Climbing Mt. Everest without oxygen: an analysis of maximal exercise during extreme hypoxia. Resp. Physiol. 52: 265-279.

West, J.B. (1986) Lactate during exercise at extreme altitude. Fed. Proc. 45: 2953-2957.

West, J.B. (1990) Limiting factors for exercise at extreme altitudes. Clin. Physiol. 10: 265-272.

West, J.B., Boyer, S.J., Graber, D.J., Hackett, P.H., Maret, K.H., Milledge, J.S., Peters, R.M., Jr., Pizzo, C.J., Samaja, M., Sarnquist, F.H., Schoene, R.B., and Winslow, R.M. (1983a) Maximal exercise at extreme altitude on Mount Everest. J. Appl. Physiol. 55(3): 688-698.

West, J.B., Hackett, P.H., Maret, K.H., Milledge, J.S., Peters, R.M., Jr., Pizzo, C.J., and Winslow, R.M. (1983b) Pulmonary gas exchange on the summit of Mount Everest. J. Appl. Physiol. 55(3): 678-687.

Winslow, R.M., Monge, C.C., Statham, N.J., Gibson, C.G., Charache, S., Whittenbury, J., Moran, O., and Berger, R.L. (1981) Variability of oxygen affinity of blood: human subjects native to high altitude. J. Appl. Physiol. Respirat. Environ. Exercise Physiol. 51: 1411-1416.

Winslow, R.M., and Monge, C.C. (1987) Hypoxia, polycythemia, and chronic mountain sickness. John Hopkins University Press, Baltimore.

Winslow, R.M., Chapman, K.W., Gibson, C.C., Samaja, M., and Monge, C.C. (1989) Different haematogic responses to hypoxia in Sherpa and Quechua Indians. J. Appl. Physiol. 66: 1561-1569.

Wolfel, E.E., Groves, B.M., Brooks, G.A., Butterfield, G., Mazzeo, R.S., Moore, L.G., Sutton, J.R., Bender, P.R., Dalms, T.E., McCullough, R.E., McCullough, R.G., Huang, S.Y., Sun, S.F., Grover, R.F., Hultgren, H.N., and Reeves, J.T. Oxygen transport during steady state submaximal exercise in chronic hypoxia. J. Appl. Physiol. in press.

Yoshida, T., Udo, M., Chida, M., Makiguchi, K., Ichioka, M., and Muraoka, I. (1989) Arterial blood gases, acid-base balance, and lactate and gas exchange variables during hypoxic exercise. Int. J. Sports Med. 10(4): 279-285.

Young, A.J., Evans, W.J., Cymerman, A., Pandolf, K.B., Knapik, J.J., and Maher, J.T. (1982) Sparing effect of chronic high altitude exposure on muscle glycogen utilization. J. Appl. Physiol. 52(4): 857-862.

APPENDICES

APPENDIX A:

GLOSSARY

MAXIMAL AEROBIC POWER ($\dot{V}O_2max$): the maximal amount of oxygen that an organism can be stimulated to extract from the atmosphere and then transport to and use in tissue.

PROGRESSIVE TEST: maximum amount of oxygen that can be consumed per unit of time by an individual during large muscle group activity of progressively increasing intensity that is continued until exhaustion.

MAXIMAL AEROBIC CAPACITY: the time to fatigue at a given percentage of $\dot{V}O_2max$.

APPENDIX B:

CONSENT FORM

INVESTIGATION OF HYPOXIA TRAINING ON SEA LEVEL PERFORMANCE

INFORMATION AND CONSENT FORM

The principal investigator for the project is Lori Melissa, under the supervision of Dr. Duncan MacDougall. A detailed verbal description of the procedures in the training study will be given in addition to this written information. After carefully reading the following information, please sign it if you wish to be a subject for the study.

A. PURPOSE

The purpose of this study is to examine the way in which hypoxia (decreased inspired oxygen concentrations) training results in improved sea level performance.

B. PROCEDURES

Initially, subjects will perform one-legged cycle ergometer exercise tests. Subjects will cycle with progressively increasing workloads until maximal levels are reached. During the test, measurements of ventilation, oxygen uptake, heart rate and blood lactate will be made.

Blood will be taken from the finger tip. Following one hour of rest, subjects will perform the test with the other leg.

On separate days subjects will perform single leg cycling to exhaustion at a set percentage of the leg's maximum workload. Following these tests, a small sample of muscle will be taken from the quadriceps muscle by what is known as the needle biopsy procedure.

The subjects will then begin their 8-10 weeks of cycle training. One leg will be randomly trained while breathing room air (20.93% oxygen) and the other leg will train while breathing 13.5% oxygen. The lower oxygen concentrations are accomplished by bleeding nitrogen into room air. Each leg will train for 30 minutes, 3 times a week, at a set percentage of maximum workload. During the last few weeks of training, some sessions will encompass interval work at levels set to percentages of maximum workload. After the training weeks are completed, the above pre-training movements will be made. Once again the muscle biopsy technique will be used to take a small sample of muscle tissue from each leg.

C. POSSIBLE RISKS of the NEEDLE BIOPSY PROCEDURE

This procedure involves the local injection of an anaesthetic (freezing) into the skin of the quadriceps area, after which a small (4mm) incision will be made and a small (50-100mg) piece of muscle will be removed with a special needle. After the procedure a suture will close the skin and pressure will be applied to minimize bruising. Most people report little discomfort with the procedure. It will be performed by a physician who is familiar with the technique.

Complications with the procedure are rare. However, in our experience with the athletes, less than 1 in 400-500 subjects experience a local skin infection, 1 in 30-40 have a temporary (up to 4 months) localized loss of sensation in the skin at the site and a few subjects have mild bruising around the incision for 4-5 days. There is also the very rare (one in a million) chance that you may be allergic to the local anaesthetic.

D. CONFIDENTIALITY

The data collected will be used in preparation of reports to be published in scientific journals. Subjects will not be identified by name in these reports. You will

have access to your own data when it is available, for your own interest.

E. REMUNERATION

You will receive a minimum honorarium of $350 to help compensate you for your time commitment. This project requires that subjects do not participate in any other training outside and above that of this study.

F. FREEDOM TO WITHDRAW

You are free to withdraw from the study at any time. If, after reading the above information, you are interested in participating as a subject you should read the statement below and sign in the space provided.

I HAVE READ AND UNDERSTAND THE ABOVE EXPLANATION OF THE PURPOSE AND PROCEDURES OF THE PROJECT AND AGREE TO PARTICIPATE AS A SUBJECT.

Signature: _____

Witness: _____

Date: _____

APPENDIX C:

SUBJECT CHARACTERISTICS

SUBJECT	CHARACTERISTIC		
	AGE (yrs.)	WEIGHT (kg)	$\dot{V}O_2max$ (mean) ($ml \cdot kg^{-1} \cdot min^{-1}$)
1	19	81.3	42.6
2	20	69.5	38.1
3	20	62.3	49.4
4	21	75.2	40.1
5	20	92.0	40.5
6	20	80.5	36.9
7	25	65.6	38.9
8	21	86.1	40.4
9	20	90.2	32.9
10	19	69.5	42.8

APPENDIX D:

LACTATE THRESHOLD

LACTATE THRESHOLD DATA: The corresponding power output and $\dot{V}O_2$ values at which there was an exponential rise in blood lactate concentration during pre- and post-training unilateral progressive maximal cycle ergometry tests for both the normoxically- and hypoxically-trained legs. Data given are means ± (SD).

	NORMOXIA		HYPOXIA	
	PRE	POST	PRE	POST
POWER OUTPUT (watts)	135.0 (27.39)	145.5 (27.43)	145.5 (22.42)	144.0 (30.38)
$\dot{V}O_2$ ($ml \cdot kg^{-1} min^{-1}$)	30.4 (5.08)	28.7 (4.76)	32.0 (5.08)	30.3 (3.09)

APPENDIX E:

RAW DATA

PERFORMANCE MEASURES: $\dot{V}O_2max$ AND MAC

PRE-TRAINING NORMOXIA

MEASURE	SUBJECT									
	1	2	3	4	5	6	7	8	9	10
$P.O._{max}$ (W)	180	165	180	180	180	165	135	180	150	150
$\dot{V}O_2max$ ($ml \cdot kg^{-1} \cdot min^{-1}$)	40.2	40.6	51.0	40.0	41.5	35.5	38.4	35.4	33.2	41.2
HR_{max} (bpm)	158	170	198	178	180	164	174	170	178	178
Ve_{max} ($L \cdot min^{-1}$)	102	81	125	104	108	97	105	113	112	116
$LACT_{max}$ ($mmol \cdot L^{-1}$)	7.2	8.1	7.2	6.4	8.3	5.0	8.8	5.0	6.5	6.4
MAC (min)	7.0	14.0	9.2	8.1	12.0	17.4	18.5	20.7	7.5	15.1
$\dot{V}O_2$ ($ml \cdot kg^{-1} \cdot min^{-1}$)	28.4	32.0	40.0	35.1	30.0	34.6	32.1	35.5	32.5	36.8

PRE-TRAINING HYPOXIA

MEASURE	SUBJECT									
	1	2	3	4	5	6	7	8	9	10
$P.O._{max}$ (W)	180	150	180	180	180	180	135	200	165	165
$\dot{V}O_2max$ ($ml \cdot kg^{-1} \cdot min^{-1}$)	45.0	35.5	47.8	40.2	39.4	38.3	39.5	45.4	32.6	44.5
HR_{max} (bpm)	160	188	192	182	178	170	160	182	176	186
Ve_{max} ($L \cdot min^{-1}$)	123	77	105	109	110	103	97	125	110	106
$LACT_{max}$ ($mmol \cdot L^{-1}$)	6.7	7.9	8.2	7.3	6.5	6.1	6.6	7.2	8.2	9.0
MAC (min)	6.0	11.0	4.3	10.1	13.5	18.1	8.7	5.0	17.3	9.5
$\dot{V}O_2$ ($ml \cdot kg^{-1} \cdot min^{-1}$)	35.9	35.5	41.7	31.5	29.6	32.4	36.6	40.5	24.9	42.4

PERFORMANCE MEASURES: VO_2max AND MAC

POST-TRAINING NORMOXIA

MEASURE	SUBJECT									
	1	2	3	4	5	6	7	8	9	10
$P.O._{max}$ (W)	200	165	180	180	220	220	150	220	180	150
$\dot{V}O_2max$ ($ml \cdot kg^{-1} \cdot min^{-1}$)	43.5	48.0	50.8	40.7	44.9	45.1	42.8	48.3	40.9	43.6
HR_{max} (bpm)	148	180	192	176	178	184	182	180	186	174
Ve_{max} ($L \cdot min^{-1}$)	119	89	108	114	125	130	104	126	148	97
$LACT_{max}$ ($mmol \cdot L^{-1}$)	8.3	8.2	8.3	7.0	8.3	9.1	8.2	6.8	8.9	6.1
MAC (min)	60.4	78.9	35.1	46.4	75.0	104	70.1	70.6	48.2	62.0
$\dot{V}O_2$ ($ml \cdot kg^{-1} \cdot min^{-1}$)	31.5	29.1	35.2	30.6	28.9	25.9	26.3	27.4	25.9	24.9

POST-TRAINING HYPOXIA

MEASURE	SUBJECT									
	1	2	3	4	5	6	7	8	9	10
$P.O._{max}$ (W)	180	165	200	180	200	220	150	220	180	150
$\dot{V}O_2max$ ($ml \cdot kg^{-1} \cdot min^{-1}$)	36.4	53.6	49.0	44.4	40.0	49.9	43.1	46.4	42.6	41.5
HR_{max} (bpm)	150	174	198	176	170	184	178	180	180	172
Ve_{max} ($L \cdot min^{-1}$)	120	97	104	120	118	134	106	111	142	102
$LACT_{max}$ ($mmol \cdot L^{-1}$)	7.0	9.6	8.1	7.3	7.1	8.9	11.4	6.8	9.6	10.3
MAC (min)	79.2	80.3	40.4	57.8	75.0	70.1	70.0	51.2	80.0	31.0
$\dot{V}O_2$ ($ml \cdot kg^{-1} \cdot min^{-1}$)	28.8	25.5	36.6	26.1	23.8	27.4	28.4	32.0	18.2	34.3

PHYSIOLOGICAL MEASURES AT THE SAME ABSOLUTE POWER OUTPUT (75% PRE-TRAINING MAXIMUM)

PRE-TRAINING NORMOXIA

MEASURE	SUBJECT									
	1	2	3	4	5	6	7	8	9	10
$\dot{V}O_2$ ($ml \cdot kg^{-1} \cdot min^{-1}$)	27.2	27.9	35.4	27.1	27.0	26.0	26.9	26.5	24.9	29.7
HR (bpm)	138	150	180	155	145	145	142	153	160	141
Ve ($L \cdot min^{-1}$)	53.0	49.9	67.2	54.1	52.6	59.1	45.3	51.4	74.4	60.3
LACT ($mmol \cdot L^{-1}$)	4.1	3.3	2.5	2.6	2.6	3.3	3.1	3.2	2.5	2.4

PRE-TRAINING HYPOXIA

MEASURE	SUBJECT									
	1	2	3	4	5	6	7	8	9	10
$\dot{V}O_2$ ($ml \cdot kg^{-1} \cdot min^{-1}$)	29.0	24.6	34.5	25.9	28.7	25.0	25.3	31.5	23.7	32.2
HR (bpm)	134	140	171	149	157	155	136	148	152	155
Ve ($L \cdot min^{-1}$)	58.1	31.7	55.3	57.3	62.1	51.5	45.3	50.9	68.2	61.4
LACT ($mmol \cdot L^{-1}$)	4.7	2.5	3.9	3.4	2.8	2.8	2.3	2.8	3.6	3.3

PHYSIOLOGICAL MEASURES AT THE SAME ABSOLUTE POWER OUTPUT (75% PRE-TRAINING MAXIMUM)

POST-TRAINING NORMOXIA

MEASURE	SUBJECT									
	1	2	3	4	5	6	7	8	9	10
$\dot{V}O_2$ ($ml \cdot kg^{-1} \cdot min^{-1}$)	24.6	34.0	33.1	22.4	25.3	26.5	27.1	28.2	22.3	27.4
HR (bpm)	122	142	167	129	141	147	136	131	133	134
Ve ($L \cdot min^{-1}$)	44.1	41.3	49.1	46.9	52.4	47.2	47.9	51.7	43.6	51.1
LACT ($mmol \cdot L^{-1}$)	4.3	3.9	3.7	3.6	3.1	2.3	3.6	3.1	3.6	3.1

POST-TRAINING HYPOXIA

MEASURE	SUBJECT									
	1	2	3	4	5	6	7	8	9	10
$\dot{V}O_2$ ($ml \cdot kg^{-1} \cdot min^{-1}$)	22.7	31.3	31.9	28.4	24.7	28.3	28.0	31.4	25.3	30.0
HR (bpm)	111	127	169	149	113	150	136	148	142	142
Ve ($L \cdot min^{-1}$)	36.8	45.5	48.8	58.6	50.2	52.9	50.1	55.5	56.6	47.7
LACT ($mmol \cdot L^{-1}$)	1.4	3.5	3.0	4.4	2.8	3.3	3.1	3.4	3.5	5.2

MORPHOMETRIC DATA

PRE-TRAINING

MEASURE	SUBJECT									
	1	2	3	4	5	6	7	8	9	10
CAP./F	0.9	-	-	-	1.5	2.0	1.0	0.9	2.8	0.8
CAP. D (per mm^2)	103	-	-	-	135	110	71	142	177	123
TYPE I (um^2)	43.5	54.9	-	55.0	77.7	77.5	88.0	34.9	61.2	39.4
TYPE II (um^2)	70.9	61.2	-	63.8	72.9	101	73.4	49.7	87.0	46.1
%TYPE I	31.1	33.6	-	49.3	50.0	28.8	38.2	60.2	30.0	44.3

POST-TRAINING NORMOXIA

MEASURE	SUBJECT									
	1	2	3	4	5	6	7	8	9	10
CAP./F	1.3	1.5	1.7	1.4	2.1	1.6	1.9	1.1	1.4	2.0
CAP.D (per mm^2)	116	142	200	135	97	139	152	132	132	139
TYPE I (um^2)	63.5	57.3	42.3	50.5	68.5	71.2	65.9	52.5	61.1	57.6
TYPE II (um^2)	69.9	71.2	54.7	65.6	145	84.4	73.8	84.5	84.0	104
%TYPE I	53.3	46.6	54.0	50.5	24.5	33.3	23.3	70.5	40.0	31.8

POST-TRAINING HYPOXIA

MEASURE	SUBJECT									
	1	2	3	4	5	6	7	8	9	10
CAP./F	1.4	1.6	1.6	1.1	1.7	1.2	1.3	1.3	2.4	3.2
CAP.D (per mm^2)	158	213	135	155	142	84	116	113	200	132
TYPE I (um^2)	50.9	37.6	52.6	46.6	59.3	67.4	73.0	53.4	51.3	90.4
TYPE II (um^2)	57.6	47.3	95.5	67.9	102	116	73.2	51.2	83.4	125
%TYPE I	59.2	41.1	52.5	49.0	50.0	33.9	29.2	59.5	42.0	40.0

ULTRAMUSCULAR STRUCTURES

PRE-TRAINING

MEASURE	SUBJECT									
	1	2	3	4	5	6	7	8	9	10
MYOF	128	117	130	135	133	125	132	126	124	142
CYTO	32	41	28	27	27	33	26	33	34	17
MITO	7	8	9	6	6	8	9	8	8	8
LIPID	1	2	1	0	2	2	1	1	2	1

POST-TRAINING NORMOXIA

MEASURE	SUBJECT									
	1	2	3	4	5	6	7	8	9	10
MYOF	121	139	123	142	140	130	123	134	126	142
CYTO	36	21	33	17	20	32	35	23	34	18
MITO	9	7	11	9	8	5	8	11	7	6
LIPID	2	1	1	0	0	1	2	0	1	2

POST-TRAINING HYPOXIA

MEASURE	SUBJECT									
	1	2	3	4	5	6	7	8	9	10
MYOF	130	125	132	120	127	132	125	137	128	129
CYTO	26	31	27	41	32	26	31	24	30	29
MITO	10	10	8	6	8	9	9	6	9	9
LIPID	2	2	1	1	1	1	3	1	1	1

BIOCHEMICAL DATA (ENZYME ACTIVITY)

PRE-TRAINING

MEASURE	SUBJECT									
	1	2	3	4	5	6	7	8	9	10
CS ($mmol \cdot hr^{-1} \cdot g^{-1}$)	0.54	0.10	0.72	0.66	0.61	0.55	0.76	0.53	1.04	1.08
SDH ($umol \cdot hr^{-1} \cdot g^{-1}$)	65.7	107	135	127	148	88.4	140	116	116	114
PFK ($mmol \cdot hr^{-1} \cdot g^{-1}$)	1.16	2.22	2.55	2.82	1.91	2.65	2.21	2.36	2.25	3.01

POST-TRAINING NORMOXIA

MEASURE	SUBJECT									
	1	2	3	4	5	6	7	8	9	10
CS ($mmol \cdot hr^{-1} \cdot g^{-1}$)	1.52	0.57	1.20	0.82	0.15	0.61	1.05	0.92	1.26	1.28
SDH ($umol \cdot hr^{-1} \cdot g^{-1}$)	182	168	214	166	111	115	242	205	141	237
PFK ($mmol \cdot hr^{-1} \cdot g^{-1}$)	2.57	2.35	3.16	3.19	1.96	2.79	3.13	3.33	2.52	3.26

POST-TRAINING HYPOXIA

MEASURE	SUBJECT									
	1	2	3	4	5	6	7	8	9	10
CS ($mmol \cdot hr^{-1} \cdot g^{-1}$)	1.65	0.68	1.31	0.85	0.60	1.07	1.10	0.93	1.49	1.31
SDH ($umol \cdot hr^{-1} \cdot g^{-1}$)	203	176	207	161	183	165	183	227	216	163
PFK ($mmol \cdot hr^{-1} \cdot g^{-1}$)	2.53	3.24	3.51	3.79	2.19	3.65	2.66	2.87	3.18	2.96

LACTATE THRESHOLD

PRE-TRAINING NORMOXIA

MEASURE	SUBJECT									
	1	2	3	4	5	6	7	8	9	10
P.O. (W)	105	135	105	135	180	135	135	180	135	105
$\dot{V}O_2$ ($ml \cdot kg^{-1} min^{-1}$)	23	37	27	27	40	27	30	33	30	30

PRE-TRAINING HYPOXIA

MEASURE	SUBJECT									
	1	2	3	4	5	6	7	8	9	10
P.O. (W)	135	135	105	135	165	165	165	180	135	135
$\dot{V}O_2$ ($ml \cdot kg^{-1} min^{-1}$)	29	42	27	32	34	30	32	37	24	33

POST-TRAINING NORMOXIA

MEASURE	SUBJECT									
	1	2	3	4	5	6	7	8	9	10
P.O. (W)	165	135	135	135	180	165	105	180	150	105
$\dot{V}O_2$ ($ml \cdot kg^{-1} min^{-1}$)	25	35	33	22	33	32	26	31	28	22

POST-TRAINING HYPOXIA

MEASURE	SUBJECT									
	1	2	3	4	5	6	7	8	9	10
P.O. (W)	180	105	135	135	165	165	105	180	165	105
$\dot{V}O_2$ ($ml \cdot kg^{-1} min^{-1}$)	30	29	32	27	29	37	28	31	33	27

APPENDIX F:

SUMMARY OF ANOVA TABLES

POWER OUTPUT$_{max}$

EFFECT	df EFFECT	MS EFFECT	df ERROR	MS ERROR	F	p-level
Training(T)	1	2722.5	9	264.2	10.3	0.01
Condition(C)	1	22.5	9	66.9	0.34	-
T x C	1	122.5	9	55.8	2.19	0.17

$\dot{V}O_2max$

EFFECT	df EFFECT	MS EFFECT	df ERROR	MS ERROR	F	p-level
Training(T)	1	204.0	9	25.7	7.93	0.02
Condition(C)	1	2.3	9	5.9	0.38	-
T x C	1	4.0	9	12.3	0.33	-

HR$_{max}$

EFFECT	df EFFECT	MS EFFECT	df ERROR	MS ERROR	F	p-level
Training(T)	1	10.0	9	80.7	0.12	-
Condition(C)	1	1.6	9	24.3	0.07	-
T x C	1	48.4	9	27.1	1.79	0.21

Ve_{max}

EFFECT	df EFFECT	MS EFFECT	df ERROR	MS ERROR	F	p-level
Training(T)	1	865.6	9	231.5	3.74	0.08
Condition(C)	1	0.4	9	39.2	0.01	-
T x C	1	1.5	9	55.9	0.27	-

$LACT_{max}$

EFFECT	df EFFECT	MS EFFECT	df ERROR	MS ERROR	F	p-level
Training(T)	1	12.9	9	1.1	12.2	0.01
Condition(C)	1	3.4	9	1.7	2.03	0.19
T x C	1	0.1	9	1.2	0.09	-

MAC

EFFECT	df EFFECT	MS EFFECT	df ERROR	MS ERROR	F	p-level
Training(T)	1	27661.3	9	180.0	153	0.00
Condition(C)	1	42.4	9	161.0	0.26	-
T x C	1	2.9	9	82.8	0.03	-

$\dot{V}O_2$ at 5 min. in MAC

EFFECT	df EFFECT	MS EFFECT	df ERROR	MS ERROR	F	p-level
Training(T)	1	367.8	9	23.1	15.9	0.00
Condition(C)	1	2.2	9	12.2	0.18	-
T x C	1	8.5	9	30.5	0.28	-

$\dot{V}O_2$ (75% Absolute P.O.)

EFFECT	df EFFECT	MS EFFECT	df ERROR	MS ERROR	F	p-level
Training(T)	1	17.1	9	28.8	0.59	-
Condition(C)	1	1.2	9	15.7	0.08	-
T x C	1	2.8	9	17.9	0.16	-

HR (75% Absolute P.O.)

EFFECT	df EFFECT	MS EFFECT	df ERROR	MS ERROR	F	p-level
Training(T)	1	1404.2	9	-	7.57	0.01
Condition(C)	1	1.2	9	-	0.01	0.94
T x C	1	7.2	9	-	0.04	0.84

Ve (75% Absolute P.O.)

EFFECT	df EFFECT	MS EFFECT	df ERROR	MS ERROR	F	p-level
Training(T)	1	429.7	9	70.0	6.14	0.03
Condition(C)	1	0.1	9	19.4	0.01	-
T x C	1	70.4	9	36.7	1.92	0.20

LACTATE (75% Absolute P.O.)

EFFECT	df EFFECT	MS EFFECT	df ERROR	MS ERROR	F	p-level
Training(T)	1	1.0	9	0.6	1.53	0.25
Condition(C)	1	0.1	9	0.6	0.14	-
T x C	1	0.3	9	0.6	0.42	-

CAPILLARY/FIBER RATIO

EFFECT	df EFFECT	MS EFFECT	df ERROR	MS ERROR	F	p-level
Training(T)	1	0.6	6	0.8	0.81	-
Condition(C)	1	0.0	6	0.1	0.33	-
T x C	1	0.0	6	0.1	0.33	-

CAPILLARY DENSITY

EFFECT	df EFFECT	MS EFFECT	df ERROR	MS ERROR	F	p-level
Training(T)	1	603.6	6	903.6	0.67	-
Condition(C)	1	51.6	6	539.1	0.10	-
T x C	1	51.6	6	539.1	0.10	-

TYPE I AREA

EFFECT	df EFFECT	MS EFFECT	df ERROR	MS ERROR	F	p-level
Training(T)	1	5.5	8	304.1	0.02	-
Condition(C)	1	9.2	8	57.7	0.16	-
T x C	1	9.2	8	57.7	0.16	-

TYPE II AREA

EFFECT	df EFFECT	MS EFFECT	df ERROR	MS ERROR	F	p-level
Training(T)	1	1791.4	8	729.1	2.46	0.15
Condition(C)	1	94.5	8	146.3	0.65	-
T x C	1	94.5	8	146.3	0.65	-

FIBER TYPE DISTRIBUTION

EFFECT	df EFFECT	MS EFFECT	df ERROR	MS ERROR	F	p-level
Training(T)	1	61.1	8	152.7	0.40	-
Condition(C)	1	24.9	8	26.4	0.94	-
T x C	1	24.9	8	26.4	0.94	-

MYOFIBRIL

EFFECT	df EFFECT	MS EFFECT	df ERROR	MS ERROR	F	p-level
Training(T)	1	8.1	9	78.3	0.10	-
Condition(C)	1	36.1	9	35.0	1.03	0.34
T x C	1	36.1	9	35.0	1.03	0.34

CYTOPLASM

EFFECT	df EFFECT	MS EFFECT	df ERROR	MS ERROR	F	p-level
Training(T)	1	22.5	9	46.0	0.49	-
Condition(C)	1	19.6	9	29.7	0.66	-
T x C	1	19.6	9	29.7	0.66	-

MITOCHONDRIA

EFFECT	df EFFECT	MS EFFECT	df ERROR	MS ERROR	F	p-level
Training(T)	1	3.0	9	1.3	2.3	0.16
Condition(C)	1	0.2	9	2.3	0.10	-
T x C	1	0.2	9	2.3	0.10	-

LIPID

EFFECT	df EFFECT	MS EFFECT	df ERROR	MS ERROR	F	p-level
Training(T)	1	0.1	9	0.9	0.11	-
Condition(C)	1	0.4	9	0.1	3.28	0.10
T x C	1	0.4	9	0.1	3.28	0.10

CITRATE SYNTHASE

EFFECT	df EFFECT	MS EFFECT	df ERROR	MS ERROR	F	p-level
Training(T)	1	1.3	9	0.1	12.3	0.01
Condition(C)	1	0.1	9	0.0	9.14	0.01
T x C	1	0.1	9	0.0	9.14	0.01

SDH

EFFECT	df EFFECT	MS EFFECT	df ERROR	MS ERROR	F	p-level
Training(T)	1	45414.1	9	1219.0	37.3	0.00
Condition(C)	1	278.8	9	615.7	0.45	-
T x C	1	278.8	9	615.7	0.45	-

PFK

EFFECT	df EFFECT	MS EFFECT	df ERROR	MS ERROR	F	p-level
Training(T)	1	4.0	9	0.1	31.7	0.00
Condition(C)	1	0.1	9	0.1	2.04	0.18
T x C	1	0.1	9	0.1	2.04	0.18

LACTATE THRESHOLD (P.O.)

EFFECT	df EFFECT	MS EFFECT	df ERROR	MS ERROR	F	p-level
Training(T)	1	202.5	9	540.0	0.38	-
Condition(C)	1	202.5	9	165.0	1.23	0.30
T x C	1	359.9	9	72.5	4.97	0.06

LACTATE THRESHOLD ($\dot{V}O_2$)

EFFECT	df EFFECT	MS EFFECT	df ERROR	MS ERROR	F	p-level
Training(T)	1	28.9	9	27.8	1.04	0.34
Condition(C)	1	25.6	9	10.6	2.42	0.15
T x C	1	0.0	9	0.0	-	-