THE EFFECT OF NARCOSIS ON AEROBIC WORK PERFORMANCE

by

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Abstract

Previous findings of a narcosis-induced reduction in heat production during cold exposure have been attributed to a reduced shivering response, as reflected in oxygen uptake (Mekjavic and Sundberg, 1992) however, the possibility of reduced oxygen utilization ($\dot{V}O_2$) by the muscles could not be excluded. Accordingly, the present study examined whether narcosis affects $\dot{V}O_2$ during incremental load exercise to exhaustion (MAX) and submaximal steady state exercise (SUBMAX). Nine male subjects participated in both the MAX and SUBMAX trials on two separate occasions. On one occasion they inspired room air (AIR) while on the other they inspired a normoxic mixture containing 30% N_2O (N_2O). In the MAX trials, work rate was increased by 30 $W.min^{-1}$ until the cadence of 60 rpm could no longer be maintained. In the SUBMAX trials, subjects exercised for 20 min at 50% of maximal workrate (Wmax) as determined in the MAX-AIR trial. At minute intervals, measurements were made of esophageal temperature (Tes), sweating rate (Esw), forehead cutaneous blood perfusion (SkBP), heat flux ($\dot{Q}$), skin temperature (Tsk), heart rate (HR), ventilation ($\dot{V}_I$) and $\dot{V}O_2$. In addition, venous blood was analyzed for lactate in the SUBMAX trials. Though subjects attained the same Wmax in the MAX-AIR and N_2O trials, $\dot{V}O_{2max}$ ($\pm$ SE) was significantly higher during N_2O (58.9 ± 3.1 $ml.kg^{-1}.min^{-1}$) compared to the AIR condition (55.0 ± 2.4 $ml.kg^{-1}.min^{-1}$). However, the $\dot{V}O_2$-relative work rate relation was similar during both MAX-AIR and MAX-N_2O at submaximal work rates. There was no statistically significant difference in $\dot{V}O_2$ and plasma lactate concentration between the AIR and N_2O conditions of the SUBMAX trials. Though there was no difference in carbon dioxide production ($\dot{V}CO_2$) between the MAX trials, the respiratory exchange ratio (RER) was significantly lower during N_2O (1.15 ± 0.02 compared to 1.25 ± 0.03). There were no significant differences in the responses of Tes, Esw, SkBP, $\dot{Q}$, Tsk, HR and $\dot{V}_I$ between the AIR and N_2O trials of the MAX and SUBMAX tests. These results suggest that narcosis, induced by 30% nitrous oxide, may increase oxygen extraction by the cells when muscles are

working maximally, resulting in improved oxygen utilization without affecting the thermal balance or exercise thermoregulation mechanisms of the body. Furthermore, the narcosis-induced reduction in $\dot{V}O_2$ observed during cold water immersion can be interpreted as a reduction in the shivering response rather than decreased oxygen utilization by the muscles.

Dedication

My mother, for setting me free

My father, for letting me go

Mrs. Taylor, for letting me daydream in class

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BACKGROUND

This study investigated the effects of nitrous oxide, an anesthetic, on aerobic performance in humans. Many studies have shown deleterious effects of inert gas narcosis on cognitive and psychomotor performance in divers at increased ambient pressure and when the nitrogen fraction of air is replaced with nitrous oxide. The few studies which have explored the effects of narcosis on physical performance, have produced variable results and were complicated by the effects of increased PO_2 and hydrostatic pressure. The effects of increased PO_2 and hydrostatic pressure on the body may be controlled for by using an anaesthetic gas such as nitrous oxide (N_2O) which exhibits narcotic effects at atmospheric pressure similar to those experienced during nitrogen narcosis (Fowler *et al.*, 1985).

A brief review of aerobic metabolism of skeletal muscle and fibre recruitment is presented followed by a review of inert gas narcosis and the known effects of increased ambient pressure and anesthesia on physical performance in humans.

1. Aerobic Metabolism of Skeletal Muscle

At rest, approximately 30% of the basal metabolic rate is accounted for by skeletal muscle oxygen uptake whereas during maximal work, muscle metabolism accounts for almost 87% of total oxygen consumption (Hochachka and Somero, 1984). It is therefore not surprising that the tissue partial pressure of oxygen (PO_2) as well as the PO_2 in the venous blood from the muscles drops to nearly zero (Finch and Lenfant, 1972) when the O_2 requirements of the muscles are being met. The increase in $\dot{V}O_2$ during exercise is partially made possible by an increase in sympathetic outflow from adrenergic and noradrenergic nerve endings, thus affecting vasomotor tone to redirect blood flow to where the metabolic demand is greatest. The increase in blood flow and

thus oxygen delivery (DO_2) to the cells increases $\dot{V}O_2$ and the rate of oxidative phosphorylation (Balaban, 1990). Therefore, it seems reasonable to suggest that a decrease in blood flow would decrease the energy available for muscle contraction.

a) Regulation of respiration by muscle mitochondria

During aerobic exercise, oxidative phosphorylation is the major metabolic process by which ATP is resynthesized. The rate of ATP synthesis is potentially modulated by concentrations of substrate, ADP, Pi, O_2, NADH and Ca^{2+} (Balaban, 1990; Moreno-Sanchez and Torres-Marquez, 1991).

Reducing equivalents in the form of pyruvate, fats and other reduced carbon chains are transported from the cytosol through the inner and outer mitochondrial membranes into the matrix where they are oxidized by dehydrogenases in Beta-oxidation and Kreb's cycle. The NADH or FADH produced as a result of the enzymatic dehydrogenation of cycle intermediates, pass electrons to the cytochromes of the electron transport chain located in the inner mitochondrial membrane. A potential regulatory site of oxidative phosphorylation may be the net delivery of reducing equivalents from the cytosol to the cytochrome chain (Erecinska and Wilson, 1982). ADP is phosphorylated to form ATP as the electrons are passed down the chain to oxygen, the final electron acceptor. To increase ATP synthesis, a proportional increase in reducing equivalent delivery to the cytochrome chain is required. As mitochondrial respiration increases, the demand for reducing equivalents and thus glycolytic activity increase (Graham and Saltin, 1989).

Negative feedback from ATPase activity, which depends on cytosolic concentrations of ADP and Pi, can potentially regulate the rate of oxidative phosphorylation in skeletal muscle (Balaban, 1990). According to this hypothesis, an adjustment of the cytosolic ADP and Pi concentrations

with a change in work regulates the rate of oxidative phosphorylation (Chance and Williams, 1956). In highly aerobic tissues such as the heart, kidney and brain, large variations in ADP and Pi are not observed with increasing work rates, suggesting that in these tissues, cytosolic ADP and Pi play a small role in the regulation of oxidative phosphorylation (Balaban, 1990; Moreno-Sanchez and Torres-Marquez, 1991), perhaps as a fine feedback control (Balaban, 1990). However, ^{31}P-NMR studies on skeletal muscle have demonstrated large variations in ADP and Pi depending on the changes in work output, suggesting that feedback from changes in cytosolic concentrations of the ATP hydrolysis products may play a large role in regulating oxidative phosphorylation in skeletal muscle (Balaban, 1990; Nioka *et al.*, 1992).

Augmented NADH concentrations can result in an increase in the maximum rate of oxidative phosphorylation at given concentrations of ADP and Pi suggesting that an increase in NADH by increasing substrate supply may be a mechanism for stimulating ATP synthesis without significant changes in ADP and Pi concentrations (Balaban, 1990). Decreased levels of NADH due to decreased production of substrates in the cytosol or decreased transport of substrates into the mitochondria, may act to decrease oxidative phosphorylation and thus energy production.

Hansford (1985) suggested that elevated cytosolic Ca^{2+} is the signal used to stimulate increases in oxidative phosphorylation through activation of Ca^{2+} sensitive dehydrogenases in the mitochondria. A blockage of Ca^{2+} transport into the mitochondrial matrix may decrease oxidative phosphorylation by preventing the hormonal activation of pyruvate dehydrogenase thus reducing the conversion of pyruvate to acetyl-CoA (McMillin and Madden, 1989). Decreased Ca^{2+} activation of other dehydrogenases involved in Krebs cycle would also decrease the $NADH/NAD^+$ ratio and result in decreased oxidative phosphorylation. Increased cytosolic Ca^{2+} due to hormonally or electrically stimulated release from the sarcoplasmic reticulum speeds up the rate of ATP hydrolysis by the myosin ATPase, increasing muscle contractility (Armstrong, 1976). The Ca^{2+} transport system of the inner mitochondrial membrane is thought to act as a system of

relaying changes in concentrations of cytosolic Ca^{2+} to the mitochondrial matrix (Hansford, 1985). The role of cytosolic Ca^{2+} in respiratory stimulation may be to act as an extramitochondrial signal to allow additional dehydrogenase control by the mitochondria and override feedback regulation by NADH and ATP (McMillin and Madden, 1989).

Oxygen availability within the mitochondria may not limit the rate of oxidative phosphorylation under normal conditions (Stainsby *et al.*, 1989; Balaban, 1990; Honig *et al.*, 1992) despite findings that at high $\dot{V}O_2$, the cell PO_2 drops to less than 5 Torr (Honig *et al.*, 1992) suggesting that tissue hypoxia occurs during heavy exercise. Graham and Saltin (1989) investigated whether $\dot{V}O_2$ is limited by the availability of oxygen by directly assessing the mitochondrial redox (NAD^+/NADH) state based on measurements of ammonia, alpha-ketoglutarate and glutamate. They observed that despite high levels of muscle lactate accumulation following exercise at 75 and 100% $\dot{V}O_{2max}$, the redox state rose 300%. They suggested that the increased availability of mitochondrial NAD^+ to the various dehydrogenases should serve to enhance oxidative metabolism and therefore oxygen consumption. Stainsby *et al.* (1989) assessed the redox state of cytochrome aa_3 (the enzyme complex responsible for the reduction of oxygen to water using reducing equivalents provided by the cytochrome chain) during contractions of muscles *in situ* with free flow and normoxia. They observed that the cytochrome aa_3 became 10 - 20% more oxidized during repetitive isotonic twitch and tetanic contractions suggesting that oxygen availability was adequate under these conditions. These experiments agree with a recent study relating gradients in PO_2 within red muscle to the dependence of cell metabolism on oxygen availability, where it was suggested that a low O_2 drive on electron transport is compensated by increased phosphorylation and redox drives (Honig *et al.*, 1992).

b) Substrate utilization during aerobic exercise

Though the major source of ATP during submaximal exercise is through oxidative phosphorylation, anaerobic glycolysis and muscle stores of ATP and phosphocreatine (PC) also contribute at the start of exercise or when there is a significant change in workload, until a steady state is reached (Lewis and Haller, 1989). Once a steady state is achieved, the rate of cellular respiration is not only dependent on the rate at which oxygen can be consumed but also on the efficiency of substrate utilization.

The choice of fuel used by the exercising muscles and whether it is obtained from exogenous (blood glucose and free fatty acids, FFA) or endogenous (glycogen and muscle triglycerides) sources is related to the intensity and duration of exercise. At rest, the respiratory quotient (RQ) is 0.7 indicating that FFAs are the predominant fuel for muscle while the major site of glucose consumption in this state is the brain (Wahren, 1977). At low to moderate work intensities, oxidation of exogenous blood glucose increases so that at exercise intensities below 50% $\dot{V}O_{2max}$, oxidation of blood glucose and plasma FFA predominates. However, above 50% $\dot{V}O_{2max}$ there is an increasing demand placed on endogenous glycogen stores while utilization of exogenous glucose decreases despite increased glucose uptake by the exercising legs (Green and Patla, 1992). In trained athletes, muscle glycogen utilization is lower than in untrained individuals during moderate prolonged exercise (approximately 65% $\dot{V}O_{2max}$) while energy derived from FFA is greater than 50% (Hurley *et al.*, 1986). This alteration in substrate utilization from the untrained to the trained state, has been accounted for by an increase in the mobilization of muscle triglyceride stores (Hurley *et al.*, 1986).

During maximal exercise, energy metabolism relies almost exclusively on utilization of endogenous glycogen stores due to the slower rate of FFA oxidation compared to carbohydrate oxidation (Lewis and Haller, 1989). The point of fatigue has been related to glycogen depletion of the active muscles (Callow *et al.*, 1986) following 1 to 2 hours of exercise at 70 - 80%

$\dot{V}O_{2max}$ (Hermansen *et al.*, 1967; Lewis and Haller, 1989). However, fatigue does not appear to be related to glycogen depletion during progressive exercise to $\dot{V}O_{2max}$ given that only 40 - 50% of the muscle glycogen is used (Green and Patla, 1992).

An increased rate of substrate transport is required during exercise to provide muscle mitochondria with reducing equivalents at a rate to meet cell needs. Glucose transport from the blood into the muscle cell is thought to occur via facilitated diffusion involving at least two membrane bound carriers, GLUT 1 and GLUT 4, the expression of which may be modified by insulin levels (Klip and Paquet, 1990). GLUT 4 in adipocytes and skeletal muscle seems to be responsible for most of the insulin dependent movement of glucose. Skeletal muscle contractile activity, in the absence of insulin, stimulates an increase in the number of glucose transporters in the sarcolemma. However, the insulin and contraction stimulated glucose uptake capacity differs among skeletal muscle fibre types depending on the density of GLUT 4 transporters (Henriksen *et al.*, 1990; Hoppeler and Billeter, 1991). GLUT 1 transporters, found mainly in the brain and kidney epithelial cells, are thought to be responsible for the basal rate of glucose transport in the absence of stimulation (Klip and Paquet, 1990).

FFA transport from blood into the muscle cell is thought to depend on its concentration in the plasma and occur via simple diffusion (Gollnick, 1977; Callow *et al.*, 1986) however, it has often been observed that the concentration gradient is outward from the muscle (Gorski, 1992) suggesting that the concentration gradient does not primarily determine FFA movement. It has been proposed that due to the action of a lipase on muscle triglyceride stores, there is a local increase in FFA concentration immediately surrounding the muscle cells, thus creating a gradient for inward FFA movement (Gorski, 1992). The mechanism of FFA transport into the cell remains hypothetical however, the process may involve a membrane bound fatty acid binding protein (FABP). Intracellular FABPs have been identified to play a significant role in the transport of FFAs to the mitochondria by increasing the solubility of the FFA (Hoppeler and Billeter, 1991). Chronic electrostimulation of a rat mixed muscle has been shown to increase the

FABP level (Kaufmann *et al.*, 1989) suggesting that muscular contraction may trigger an increase in intracellular FFA transporters.

c) Limitations to aerobic performance

Aerobic performance is limited by both the maximum aerobic power ($\dot{V}O_{2max}$) or the rate at which oxygen can be consumed and by the efficiency with which the task is performed, this last criteria being of greater significance in highly conditioned athletes (Whipp and Wasserman, 1969). The O_2 cost of exercise is affected by the proportion of lipid to carbohydrate that is oxidized. Since 10% less O_2 is required to synthesize a mole of ATP from glycogen than from FFA (Lewis and Haller, 1989), athletes who are able to oxidize more FFA during prolonged submaximal exercise will spare glycogen reserves, increasing the time to fatigue. Physiological factors such as ventilatory capacity, cardiac output, the distribution of blood flow to active tissues, the capacity of blood to carry O_2 and the amount of O_2 extracted by the tissues also contribute to efficiency and limit aerobic power (MacDougall *et al.*, 1991).

As exercise intensity increases there is an increased involvement of anaerobic metabolism. As the rate of glycogen breakdown exceeds the capacity of aerobic metabolism to keep up with pyruvic acid production, there is an increase in lactic acid production and accumulation occurs. The concentration of lactate in the blood reflects the difference between the amount being produced and released into the blood and the amount being used as fuel for aerobic reactions by the exercising muscles or other tissues (MacDougall *et al.*, 1991).

During submaximal exercise, the respiratory exchange ratio (RER) indicates the relative contribution of carbohydrate and fatty acid metabolism to energy production. However, during maximal aerobic exercise, the RER is not a good indicator of the proportion of fuels utilized due to the rise in plasma lactate causing excess CO_2 production (Jones *et al.*, 1980).

2. Muscle fibre types and their recruitment

There is a large variation in the proportion of fibre types found in human muscle due to differences in genotype and/or activity level (Simoneau and Bouchard, 1989). For example, the *vastus lateralis* muscle, which has been extensively studied, shows a wide range of slow and fast-twitch fibre distributions among individuals (15 - 85% ST within a population of 270 subjects; Simoneau and Bouchard, 1989) with reported average values of approximately 50% each of slow and fast-twitch fibre types (Saltin and Gollnick, 1983; Simoneau and Bouchard, 1989).

The twitch property of muscles is determined by the isoforms of the contractile proteins (i.e. actin, myosin, tropomyosin, troponin etc.) of the individual fibres which compose the muscle. These differences affect the rate at which myosin splits ATP (Saltin and Gollnick, 1983). Myosin ATPase activity is greater in fast-twitch fibres (FT) than in slow-twitch fibres (ST). FT fibres have been divided into three subgroups based on differences in oxidative capacity however, it has been suggested that the subdivision of fibres into groups of low and high oxidative potential is artificial due to the inaccuracy of histochemical staining procedures which have been used to identify fibre types on this basis (Gollnick and Hodgson, 1986). In fact, it has been shown that there is no sharp division between the oxidative potentials of fibres but that within a given muscle, a continuum of oxidative potentials exists within the fibre types with overlap between all fibre types (Pette and Spamer, 1979; Gollnick and Hodgson, 1986).

Recruitment of FT or ST fibres normally depends upon physiological need and substrate availability (Hochachka and Somero, 1984). During aerobic exercise, ST fibres are better suited to provide ATP from oxidative metabolism due to their high mitochondrial density, greater blood supply, and large triglyceride stores. For activities lasting up to 20 min, the dominant muscle fuel

source for resynthesis of ATP is glycogen, with only a minor contribution from beta-oxidation of fats. During prolonged aerobic exercise lasting longer than 1 hour, there is an increasing contribution from triglyceride stores as glycogen becomes depleted (Hoppeler and Billeter, 1991). When a sudden large power-output is required, FT fibres are recruited to provide an immediate supply of energy from a relatively large store of ATP and PC. The greater glycogen stores of FT fibres also make them suited for short-term high intensity activities that require ATP faster than can be supplied solely by oxidative metabolism (Gollnick *et al.*, 1973a, 1973b). During intense activity, anaerobic glycolysis is the main process by which energy is supplied to the muscle.

Regardless of exercise intensity, ST fibres are usually recruited first, due to a lower threshold of activation (Henneman *et al.*, 1965). Gollnick *et al.* (1973a, 1973b) observed that the larger FT fibres are only recruited when the ST fibres become glycogen depleted and fatigue or when a greater force output is required. Glycogen depletion studies have shown that when tensions exceed 20% of the maximal voluntary contraction (MVC), there is increased FT fibre recruitment (Tesch, 1980; Saltin and Gollnick, 1983). An exception to the initial recruitment of ST fibres occurs during quick ballistic-type contractions where EMG studies have shown a reduction in the activation threshold of motor units (Saltin and Gollnick, 1983). It has been suggested that there is an exclusive pool of FT motor units that fire when muscle tension is rapidly increased or suddenly forceful (Tesch, 1980). Contrary to the conclusions of Gollnick *et al.* (1973a,1973b), Vollestad and Blom (1985) observed initial recruitment of both the ST (Type I) and the oxidative subgroup of FT fibres (Type IIA) at 43% $\dot{V}O_{2max}$. They suggested that the discrepancy between their study and others which did not observe initial FT fibre recruitment was due to differences in the sensitivities of the methods used for estimation of glycogen content.

Using changes in integrated EMG (iEMG) to indicate changes in motor-unit activation, Moritani (1990) has demonstrated an increase in motor-unit recruitment correlated with the onset of a sudden increase in $\dot{V}O_2$ after the anaerobic threshold was reached. He suggested that increased

monosynaptic reflex excitability in the spinal cord, indicated by H-wave amplitude, is due to progressive recruitment of high threshold FT-oxidative and FT-glycolytic fibres with a concomitant increase in lactate production.

Increased H^+ concentration, due to accumulation of lactate, is thought to contribute to muscular fatigue by interfering with the excitation-contraction coupling process by decreasing the amount of Ca^{2+} available to bind to troponin and inhibiting cross-bridge formation (Nakamura and Schwartz, 1972; Tesch, 1980). However, muscle fatigue has also been reported in patients lacking phosphofructokinase (PFK), a rate-limiting enzyme in anaerobic glycolysis, which according to Moritani (1990) suggests that some mechanism other than lactate accumulation is responsible for fatigue. In these patients, fatigue is likely due to the reduced availability of ATP. Trivedi and Danforth (1966) suggested that increased H^+ concentration associated with lactate accumulation may contribute to fatigue by reducing ATP production (Trivedi and Danforth, 1966) and/or by affecting excitation-contraction coupling (Nakamura and Schwartz, 1972; Tesch, 1980). However, it has been demonstrated in the cat that despite fatiguing the gastrocnemius muscle to a greater extent than the soleus muscle, there is no difference in lactate release between the two muscles (Hudlicka, 1971). Accumulation of other inhibitory metabolites, such as inorganic phosphate and ammonia may induce fatigue (Moritani, 1990).

The requirement for greater force output from the exercising muscles can be achieved by increasing the number of fibres recruited, increasing the motor unit firing rate or by a combination of these strategies (Bigland-Ritchie *et al.*, 1986). In addition, there are indications that firing may become synchronized in different motor units prior to exhaustion, causing an increase in the low frequency spectral components of the surface EMG power spectrum, particularly in the 20 - 40 Hz band (Hägg, 1992). Recruitment and firing rate are increased in response to peripheral reductions in neuromuscular propagation and/or excitation contraction coupling as the exercising muscles fatigue. The specific mechanisms causing fatigue depend on the task being performed. Factors

which can contribute to a reduced ability to generate force and movement include reduced CNS drive to the muscle, reduced supply of metabolic substrates, or local accumulation of metabolites. Bigland-Ritchie *et al.* (1986) have also suggested that impairment of the link between neuromuscular activation and actomyosin cross bridge formation may affect the contractile response.

Vollestad *et al.* (1984) and Vollestad and Blom (1985) examined changes in fibre recruitment strategy with changes in the force output required to maintain given exercise intensities on a cycle ergometer. At rest before exercise, glycogen content of the fibres from the Type II (FT) subgroup was 16% higher than in the Type I (ST) fibres. Using the glycogen depletion method to identify the pattern of fibre recruitment in the *vastus lateralis*, they observed approximately 40% motor unit activation at 43% $\dot{V}O_{2max}$ and 80 - 85% activation at 75% $\dot{V}O_{2max}$. At 43% $\dot{V}O_{2max}$, all Type I fibres and 20% Type IIA (FT oxidative) fibres had been recruited. At 75% $\dot{V}O_{2max}$ all Type I and IIA fibres were recruited. At 91% $\dot{V}O_{2max}$, glycogen depletion had also occurred in more than half the Type IIB (FT glycolytic) and Type IIAB (FT oxidative-glycolytic) fibres. The authors concluded that during submaximal exercise intensities, muscle fibre recruitment plays a major role in providing greater force output. At exercise intensities above 100% $\dot{V}O_{2max}$ they suggested that increased motor unit firing rate is reponsible for maintaining force output since 90% of the fibres in the *vastus lateralis* are recruited to provide a force output of less than 50% of the maximal peak force on the pedals of the cycle ergometer.

The results of glycogen depletion studies should be interpreted with caution given the evidence presented by McDermott *et al.* (1987). They observed that during exercise, glycogenolysis also occurs in the nonexercised muscle of rats. The authors suggested that the increase in epinephrine during exercise was likely responsible for inducing glycogenolysis in these muscles.

3. Exercise Performance in a Compressed Air Environment

Increased ambient pressure during exercise is reported to either increase $\dot{V}O_2$, at a given submaximal load (Fagraeus *et al.*, 1974), increase $\dot{V}O_{2max}$ (Eagan and Plese, 1969; Fagraeus *et al.*, 1973; Linnarsson *et al.*, 1974), decrease $\dot{V}O_{2max}$ (Cook, 1970) or produce no change in $\dot{V}O_{2max}$ (Fagraeus, 1974). The conflicting results can be explained in terms of the effects of hyperoxia, increased gas density causing ventilatory impairment and CO_2 retention, and the effects of increased PN_2.

a) The effects of hyperoxia and CO_2 retention on exercise performance.

Increased $\dot{V}O_{2max}$ was observed in subjects performing cycle ergometer exercise in air at 1.4 ATA (Fagraeus *et al.*, 1973; Linnarsson *et al.*, 1974), 2.0 and 3.0 ATA (Fagraeus *et al.*, 1973) however, with increasing pressure above 2.0 ATA up to 6.0 ATA, the degree to which $\dot{V}O_{2max}$ was improved above normobaric conditions did not change significantly (Fagraeus *et al.*, 1973). The improvement in aerobic power during hyperbaric exercise appears to be related to the increase in arterial PO_2 (P_aO_2) during hyperoxic conditions created by raising the environmental air pressure. However, oxygen delivery may not increase with increasing P_aO_2 since the increase in arterial oxygen content is offset by a decrease in blood flow to active muscles (reviewed by Welch, 1987). Due to lack of evidence of an increase in cardiac output or increase in arterio-venous difference by more than 5 - 6% during hyperoxia, it is unlikely that an increase in $\dot{V}O_{2max}$ would be greater than 6% (Welch, 1987) as observed by Fagraeus *et al.* (1973) yet Linnarsson *et al.* (1974) reports a 10% higher $\dot{V}O_{2max}$ at 1.4 ATA.

Cook (1970) controlled for the effects of hyperoxia at 2.0 and 3.0 ATA by maintaining the PO_2 of the breathing mixture at control levels during treadmill exercise to exhaustion. He observed a

decrease in $\dot{V}O_{2max}$ at 3.0 ATA to 68% of the control value. This decrease was accompanied by a reduction in ventilation to 63% of the control value at 2.0 ATA and 38% of the control value at 3.0 ATA. An increase in alveolar PCO_2 (P_ACO_2) with increased metabolic acidosis, was attributed to the ventilatory insufficiency observed at pressure, thus limiting work capacity. Cook also suggested the possibility that increased PN_2 affected neuromuscular co-ordination to cause a decrement in performance and the greater variability observed between exercising subjects at increased pressure.

When hyperbaric pressure is increased further and PO_2 of the breathing mixture is not maintained at sea level values, the beneficial effects of hyperoxia are partially offset by an increase in gas density sufficient to cause ventilatory impairment, resulting in CO_2 retention (Cook, 1970; Fagraeus, 1974; Bennett, 1982) and increased central inspiratory activity (CIA; Hesser and Lind, 1980; Hesser *et al.*, 1990). No change in $\dot{V}O_{2max}$ was observed when subjects exercised on a cycle ergometer at 3.0 and 6.0 ATA compared to control values at 1.0 ATA (Fagraeus, 1974) yet when the N_2 fraction was replaced with helium at 3.0 ATA, VO_{2max} increased significantly. Wilson and Welch (1980) observed that a greater volume but lower mass of gas is moved by exercising subjects breathing 20% O_2 / 80% He, a gas one-third the density of air at sea level, resulting in reduced work of breathing despite increased ventilation. Increased expiratory muscle activity (Hesser *et al.*, 1990) with decreased ventilation in response to increased breathing resistance when denser gases are inhaled, reduces the amount of CO_2 that can be eliminated, leading to CO_2 retention. Fagraeus (1974) observed a rise in end-tidal PCO_2 from 35 mmHg at 1.0 ATA to 56 mmHg at 6.0 ATA. In all conditions, the point of exhaustion was associated with similar lactate concentrations and varied end-tidal PCO_2 levels, which according to Fagraeus (1974) suggested that lactate levels had a greater limiting effect on maximal performance than PCO_2 or pH conditions in the active muscles. Similar results were obtained by Linnarsson *et al.* (1974) at lower pressures, where no difference was found in lactate concentration after maximal

exercise at O.68, 1.0, and 1.4 ATA (i.e. hypoxic, normoxic and hyperoxic conditions, respectively) despite differences in performance.

Few studies have been performed on the effects of increased pressure on submaximal exercise. Fagraeus et al. (1974) observed an increase in $\dot{V}O_2$ at 4.5 ATA which they suggested was due to the raised N_2 pressure in terms of increased work of breathing since $\dot{V}O_2$ was similar in air and in pure oxygen at 1.0 ATA with higher values reported in air at 4.5 ATA.

b) The effect of increased PN_2 on exercise performance

Raised PN_2 does not appear to exert a major depressant effect on the respiratory centre to reduce ventilation during exercise (Hesser and Lind, 1980; Hesser *et al.*, 1990). Hesser and Lind (1980) determined changes in central inspiratory activity (CIA) with pressure by measuring the change in ventilation per unit mouth occlusion pressure ($\dot{V}/P_{0.1}$). $P_{0.1}$ indicates the neuromuscular component of the inspiratory drive (Whitelaw *et al.*, 1975) which is necessary to generate sufficient pressure to overcome the non-elastic resistance of the lungs during inspiration (Hesser and Lind, 1980). During an incremental cycle ergometer test from 0 - 200 W over a 20 min period, $\dot{V}$ was significantly lower and $P_{0.1}$ higher at loads exceeding 100 W when the exercise was performed at 6.0 ATA (PO_2 = 1.3 ATA, PN_2 = 4.7 ATA) compared to when subjects inhaled O_2 at 1.3 ATA (Hesser and Lind, 1980). The observation that $\dot{V}/P_{0.1}$ was much lower under the hyperbaric conditions indicated higher CIA compared to control conditions. The reduced ventilatory response despite increased CIA at 6.0 ATA, suggested that the much greater PN_2 did not exert a depressant effect but rather, the increased gas density with consequent increased breathing resistance was likely responsible for the reduced ventilation and increased CIA to overcome the greater resistance (Hesser and Lind, 1980).

Hesser *et al.* (1990) have shown that there is also a shift in the Hering-Breuer threshold to a higher inspiratory volume by comparing the ratios of tidal volume and inspiratory time (V_T/T_I) obtained under normobaric and hyperbaric conditions. At 6.0 bar (approximately 6.0 ATA) the inspiratory off-switch mechanism is activated at larger V_T than in the control condition. They suggested that a raised arterial P_aCO_2 due to CO_2 retention may affect vagal lung-volume feedback by reducing pulmonary stretch receptor activity. However, Hesser *et al.* (1990) noted that this explanation did not account for the tidal volumes observed at work loads < 100 W where a significantly greater V_T was obtained at 6.0 bar despite end-tidal PCO_2 values similar to control levels. They suggested that the higher PN_2 level may interfere with neural transmission to depress the Hering-Breuer reflex. If this is the case, it is likely that as exercise approaches maximal levels where the effects of increasing P_aCO_2 become apparent there is also a continuing influence of high PN_2 levels on either afferent feedback from pulmonary receptors or neural input to the respiratory muscles.

Eiken *et al.* (1987) examined the effects of PN_2 and PO_2 on power output during high intensity exercise. Subjects performing 60 maximal unilateral knee extensions at an angular velocity of $180^{o}/s$ showed a reduction in peak torque at 6.0 ATA compared to 1.0 ATA. Eiken *et al.* (1987) suggested that raised N_2 and/or hydrostatic pressure may interfere with neural input to the working muscles or impair the excitation-contraction coupling mechanism. They observed that at increased pressure maximal force was reduced to a greater extent in subjects with a greater percentage of fast twitch fibres, suggesting that there might be selective interference of neural input to these fibres. Selective interference may be explained by the higher excitation threshold of the larger fast twitch motor units compared to the smaller motor units of slow twitch fibres, thus according to the "size principle" the fast twitch fibres are less easily recruited (Henneman *et al.*, 1965).

Examination of nitrous oxide (N_2O) studies can help to explain the neuromuscular effects of increased PN_2 since N_2O exhibits narcotic effects at atmospheric pressure similar to those experienced during nitrogen narcosis (Biersner, 1987) and is thought to operate by the same mechanism (Fowler *et al.*, 1985). N_2O studies have shown that interference occurs with synaptic transmission at the motor end plate (Thesleff, 1956; Somjen, 1967). It has been proposed that either the sensitivity of the postsynaptic membrane to neurotransmitter is diminished or that there is a reduction of neurotransmitter release (Somjen, 1967). In the study by Eiken *et al.* (1987), though selective interference of neural input to fast twitch fibres could explain individual differences between subjects, it seems unlikely that these fibres should be affected differentially from slow twitch fibres if interference with neural transmission occurs at the motor end plate. However, a decrease in oxygen uptake during aerobic exercise may be caused by an alteration in the recruitment pattern so that interference with ST fibre activation would necessitate an increase in anaerobic metabolism and thus earlier FT fibre activation to maintain a given workload

Eiken *et al.* (1987) did not measure myosin ATPase activity; however, a decrease in activity would be expected during both anaerobic and aerobic exercise if acto-myosin coupling rather than the excitation-contraction coupling mechanism is affected by increased PN_2.

4. Exercise Performance During Nitrous Oxide Narcosis

Because of the effects of hyperoxia and pressure *per se*, it is difficult to discern the effect of inert gas narcosis on aerobic performance from studies conducting comparisons between normobaric and hyperbaric air environments. If the PO_2 of the hyperbaric air is lowered to normoxic levels and nitrogen is substituted with helium, the effects of hyperoxia and increased gas density can be controlled for. An alternative method to discern the effects of narcosis alone on oxygen uptake, is to use a normoxic gas mixture containing nitrous oxide (N_2O) which at atmospheric pressure

exhibits narcotic effects similar to those experienced during hyperbaric narcosis (Hesser *et al.*, 1978; Fowler *et al.*, 1985; Biersner, 1987). The narcotic potency of N_2O to N_2 is thought to be 30:1 (Miller *et al.*, 1973) thus we can obtain information on human performance during narcosis without the confounding effects of a hyperbaric environment.

Studies on exercise performance during N_2O-narcosis appear to be nonexistent, except for the investigation by Bradley and Dickson Jr., (1976). They observed that $\dot{V}O_2$ was significantly reduced in subjects breathing air mixtures containing either 15% or 30% N_2O while at rest and during cycle ergometry exercise at a load of 400 kg-m/min (Bradley and Dickson Jr., 1976). At a heavier load (800 kg-m/min) the decrease in $\dot{V}O_2$ was not significant. The observations during moderate exercise agree with the results of Cook (1970) who also found a decrease in oxygen consumption in a hyperbaric environment when he controlled for the effects of hyperoxia. A possible mechanism which could explain these results was suggested by Passias *et al.* (1992) who observed a reduction in $\dot{V}O_2$ and shivering thermogenesis during cold-water immersion when subjects breathed 30% N_2O. They proposed that N_2O attenuates effector activity such as shivering in response to cold stimulation by depressing synaptic transmission in the central nervous system (Davis *et al.*, 1957), possibly affecting central processing and integration. During submaximal exercise, it is possible that the effector response to a given load on the muscle is attenuated, reducing recruitment of slow twitch fibres. To maintain the work-load, this decrease in aerobic metabolism would necessitate an increase in anaerobic metabolism, and thus activation of fast-twitch fibres to provide the energy required for muscular contraction.

A mechanism other than, or in addition to, altered fibre recruitment which could lead to reduced $\dot{V}O_2$ is suggested by the effects of nitrous oxide on the sympathetic nervous system. N_2O has been found to increase sympathetic outflow to the skeletal muscle vascular bed of the lower leg (Ebert and Kampine, 1989; Sellgren *et al.*, 1990) and increase plasma norepinephrine levels (Eisele, 1985; Sellgren *et al.*, 1990). Alpha-adrenergic stimulation in response to analgesic levels

of N_2O results in increased peripheral vascular resistance (Eisele, 1985). Thus, decreased perfusion of blood through the muscles due to vasoconstriction may result in decreased oxygen delivery and consequently reduced oxygen uptake by the muscles; however, reduced $\dot{V}O_2$ would not likely be apparent except during high intensity exercise approaching $\dot{V}O_{2max}$, when oxygen supply cannot keep up with aerobic demand. In the absence of sympathetic innervation, N_2O has been shown to depress contractility and reduce oxygen delivery and myocardial oxygen consumption in isolated guinea pig hearts despite increased coronary flow (Stowe *et al.*, 1990, Stowe *et al.*, 1991). This suggests that oxidative phosphorylation may be limited to some extent by arterial oxygen content (Stowe *et al.*, 1990) possibly in combination with reduced blood flow, to produce a decline in muscle work.

An effect of N_2O on the oxygen carrying capacity of the blood could also reduce $\dot{V}O_2$. Haemoglobin saturation (SO_2) has been shown to decrease from 96.3 ± 1.3 in subjects breathing 50% O_2/ 50% N_2 to 94.0 +/- 2.0% following inhalation of 50% O_2/ 50% N_2O with a 2% drop in end-expired oxygen concentration (Wilkins *et al.*, 1989). Anesthetic levels of N_2O have been shown to shift the oxyhemoglobin dissociation curve to the right, both *in vitro* (Lanza *et al.*, 1988) and *in vivo* (Smith *et al.*, 1970). A decrease in SO_2 may affect maximal oxygen uptake as shown by Powers *et al.* (1989) who observed that athletes with a low SO_2 (less than 92%) due to exercise induced hypoxemia, obtained a lower $\dot{V}O_{2max}$ during exercise at sea level than those athletes with greater $\%SO_2$. If nitrous oxide adversely effects the oxygen content of arterial blood, this could lead to decreased $\dot{V}O_2$ by the working muscles unless blood flow were to increase and compensate for the reduced carrying capacity of the blood.

Though the effects of N_2O on aerobic performance may offer some insight regarding observations made at increased ambient pressure, comparisons should be made with caution until there is more information regarding exact mechanisms of anesthetic action on physiological systems.

5. Mechanisms of Inert Gas Narcosis and Anesthetic Action

The mechanism by which volatile anesthetics or inert gases at increased pressure are thought to act on physiological systems is via a biophysical rather than a biochemical means (Featherstone and Muehlbaecher, 1963; Bennett, 1982). Those gases classified as inert are the noble gases, helium, neon, argon, krypton, xenon and radon, because their molecular structure is not altered by the usual chemical reactions. Anesthetics such as N_2O and halothane are thought to fall into this category (Bennett, 1982; Fowler *et al.*, 1985). The narcotic potency of an inert gas is a function of its nature (eg. polarizability and volume of the gas molecule; Bennett, 1982) and the ambient pressure.

There are several theories as to the exact mechanism of inert gas narcosis and anesthesia with particular attention to the effects on cellular membranes (Kety *et al.*, 1948; Thesleff, 1956; Bennett, 1982) and neural structures (Davis *et al.*, 1957; Somjen, 1967; de Jong *et al.*, 1969; Avramov and Mori, 1990; Carpenter; 1991).

a) Cellular mechanisms

There is a strong correlation between the lipid solubility of an inert gas and its narcotic potency (for review see Bennett, 1982). According to the critical volume hypothesis (Miller *et al.*, 1973), anesthesia occurs when an anesthetic agent is absorbed by the lipid membrane, causing the membrane to expand beyond a critical volume. It is possible that this expansion alters the shape of the ion transport channels, thus affecting conduction and altering neuronal transmission.

In support of the critical volume hypothesis, it has been shown that an increase in hydrostatic pressure can reverse anesthesia and narcosis (Lever *et al.*, 1971). The multi-site expansion

hypothesis (Halsey *et al.*, 1978) agrees with the critical volume hypothesis however, it states that more than one molecular site with differing physical properties may be affected and that pressure itself and anesthesia may act at different sites. According to the increased permeability hypothesis, adsorption of inert gas and anesthetic molecules by lipid monolayers causes a decrease in the lipoprotein-water interface tension resulting in a transient, reversible increase in Na^+ efflux and K^+ influx (Galey and van Nice, 1980; Bennett, 1982), thus reducing cell excitability. Thesleff (1956) suggested that the sodium carrying mechanism might be inhibited based on observations that resting membrane potential and the Na^+ concentration gradient were not altered in a stimulated sartorius nerve-skeletal muscle preparation during exposure to pentobarbital, a nonvolatile anesthetic. However, despite producing similar effects, volatile and nonvolatile anesthetics may not operate by the same mechanism. In addition, high concentrations of anesthetics appear to inhibit Na^+ conductance whereas low concentrations stimulate it (Anderson, 1972). These differential effects may be related to the degree of cell membrane expansion and thus distortion of membrane transport components, depending on the concentration and nature of the anesthetic.

b) Neuronal conduction and synaptic transmission

The reticular formation of the brainstem with extensions into the thalamus, co-ordinates sensory and motor systems as well as the level of arousal. Investigation of this area of the brain and other areas of the central nervous system (CNS) in response to N_2O and other volatile anesthetics offers insight as to the mechanism of anesthetic action.

Both N_2O and increased P_{N2} are thought to exert their effects by dissolving in the lipid portion of neuronal membranes thus affecting ion conduction and neuronal transmission (Bennett, 1982). The site of action for N_2O is likely at the synapse based on observations that the excitatory postsynaptic potential (EPSP) is reduced in response to anesthetics (Thesleff, 1956; Somjen,

1967). Multisynaptic pathways are more vulnerable than oligosynaptic pathways, as supported by Davis *et al.* (1957) who found a greater depression of evoked potentials in the multisynaptic reticular activating system in response to 77% N_2O / 23% O_2 compared to the thalamus where fewer synapses are found.

Evidence suggests that interference with synaptic transmission in the spinal cord occurs postsynaptically (Somjen, 1967), possibly due to reduced effectiveness of neurotransmitter on the postsynaptic membrane though, decreased neurotransmitter release from presynaptic terminals cannot be excluded. Inhibition may also occur due to an increase in the threshold potential (Somjen, 1967) which would agree with observations made by Thesleff (1956) on skeletal muscle membrane.

Studies have observed an effect of anesthetics on efferent output from the central nervous system (CNS), while afferent pathways appear to be unaffected (de Jong and Nace, 1967; de Jong *et al.*, 1969). De Jong and Nace (1967) investigated the effect of 75% N_2O / 25% O_2 and other inhaled anesthetics on cutaneous receptor firing and axonal conduction in cats. They observed that inhalation anesthetics did not disrupt afferent transmission in peripheral nerves or generation of impulses in cutaneous receptors.

Evidence also suggests that small diameter myelinated fibres, such as those associated with pain perception, are more vulnerable to anesthetics (de Jong *et al.*, 1969; Carpenter, 1990) than larger diameter fibres. Carpenter (1990) has shown that the amount of anesthetic required to reversibly disrupt conducted responses is inversely proportional to anesthetic nonaqueous solubility. Smaller fibres have a lower lipid content thus they would be expected to require lower anesthetic concentrations to disrupt neuronal function.

If neuronal function at the neuromuscular junction is affected by narcosis/partial anesthesia during exercise, the smaller motor-units would likely be inhibited first. FT fibres would need to be recruited to make up for the loss of ST fibre activity. Such an alteration in the recruitment of muscle fibres would increase dependence on anaerobic stores to provide the energy required to maintain a given workload. However, if there is an overall increase in the activation threshold as suggested by Thesleff (1956) then reduced recruitment of the larger FT fibres could occur during prolonged submaximal exercise, possibly compensated for by increased stimulation of ST and smaller diameter FT fibres.

INTRODUCTION

Compressed air environments induce narcosis in divers, which significantly impairs their performance (Adolfson, 1967). This narcosis has been attributed to the individual and combined effects of the elevated partial pressures of nitrogen, oxygen, and carbon dioxide (Frankenhaeuser *et al.*, 1963; Hesser *et al.*, 1978). To gain a better understanding of the manner in which divers' performance is impaired at depth, many studies have examined the effects of narcosis on the ability to perform psychomotor and cognitive tasks either in a hyperbaric environment (Fowler *et al.*, 1985; Biersner, 1987), or have simulated compressed air narcosis with inhalation anesthetics, such as nitrous oxide (N_2O), at 1 ATA (Fowler *et al.*, 1985). The narcotic potency of N_2O to N_2 is thought to be 30:1 (Miller *et al.*, 1973), thus by administering N_2O at 1 ATA, the effects of narcosis on human performance can be investigated without the confounding effects of increased ambient pressure and hyperoxia.

To date, most studies have focussed on the effect of narcosis on behaviour, but its effect on autonomic function remains to be elucidated. Recently, Mekjavic and co-workers (Mekjavic and Sundberg, 1992; Passias *et al.*, 1992; Cheung and Mekjavic, 1994; Mekjavic, *et al.*, 1994a, Mekjavic, *et al.*, 1994b) have demonstrated that narcosis, whether induced with N_2O or hyperbaric nitrogen, will significantly attenuate thermal perception and the shivering response. The inhibitory effect of narcosis on behavioural and autonomic thermoregulation was suggested to be a major contributing factor to the development of hypothermia in divers.

The observed narcosis-induced reduction in heat production during cold exposure, as reflected in oxygen uptake, may be attributable to both decreased neural input to the muscles and a decrease in oxygen utilization by the muscles. N_2O has been shown to inhibit synaptic transmission (Somjen, 1967), and increase the activation threshold of motor neurons (Somjen, 1967; de Jong *et al.*, 1969). The combined effect could be reflected in a decreased muscle work capacity, since

this may reduce activation of the larger muscle fibres. Although the attenuation of oxygen uptake is most likely primarily due to an attenuation of the shivering tremor, as confirmed by Cheung and Mekjavic (1994), the possibility of an inhibitory effect of N_2O on the mitochondria and thus oxygen utilization, as reasoned by Mekjavic and Sundberg (1992), cannot be dismissed.

The present study was designed to examine whether oxygen utilization, as reflected in the oxygen uptake, would be affected during steady-state and incremental-load exercise. It was reasoned that for a given workrate, oxygen uptake would be similar, assuming that its utilization was unaffected. Previous studies have not been unequivocal, and have concluded that hyperbaric air exposure causes both an increase (Fagraeus *et al.*, 1973) and decrease in $\dot{V}O_{2max}$ (Cook, 1970; Fagraeus, 1974). Increased $\dot{V}O_{2max}$ has been explained in terms of hyperoxia (Fagraeus *et al.*, 1973), whereas decreased $\dot{V}O_{2max}$ has been attributed to increased gas density causing ventilatory impairment and CO_2 retention (Cook, 1970; Fagraeus, 1974). Insufficient CO_2 elimination occurs when alveolar ventilation is reduced due to increased breathing resistance at raised ambient pressure. The excessive levels of CO_2 in the blood and tissues produce a metabolic acidosis that is thought to be responsible for a reduction in $\dot{V}O_{2max}$ at increased pressure (Cook, 1970). Since it has been shown that hyperoxia at normal atmospheric pressure does not increase steady-state $\dot{V}O_2$ (reviewed by Welch, 1987), increased $\dot{V}O_2$ during submaximal exercise at raised air pressure has been attributed to the increased work of breathing.

With the exception of the study by Cook (1970), the above studies did not control for the possible confounding influences of hyperoxia, CO_2 retention, and hydrostatic pressure, and it is therefore not possible to discern the magnitude of the contribution of each variable. Bradley and Dickson Jr. (1976) used normoxic gas mixtures containing either 15 or 30% N_2O to assess the effects of narcosis *per se* on aerobic performance. They observed a reduced $\dot{V}O_2$ with both mixtures while subjects were at rest and during moderate cycle ergometry exercise (400 kg-m.min^{-1}), but at a heavier load (800 kg-m.min^{-1}) the decrease in $\dot{V}O_2$ was not significant. Though they did not

discuss this finding, a possible mechanism which could explain their results would be a decrease in oxygen utilization by the mitochondria with an increase in anaerobic metabolism to maintain the work load. Any effect of N_2O on mitochondria would likely be biophysical in nature (Kety *et al.*, 1948; Bennett, 1982), since N_2O is considered inert, thus dismissing the possibility of a biochemical interaction.

In addition to examining the effect of inhalation of 30% N_2O on aerobic performance, both submaximal and maximal, the present study also investigated the effect of narcosis on heat loss mechanisms during exercise, as impairment in heat dissipation may also impair exercise thermoregulation.

METHODS

1. Subjects

Nine male subjects, all fit and engaged in regular physical activity, took part in the study. Their participation was subject to physicians' approval. The mean age, height, and weight (± SE) of the subjects were 30.6 ± 3.2 yr, 183.1 ± 1.4 cm, and 78.4 ± 2.9 kg, respectively (Table 1). Only subjects comfortable breathing the nitrous oxide mixture were accepted for participation. Each subject performed two maximal and two submaximal exercise trials with a minimum of one week separating the trials.

2. Protocol

The trials were conducted with subjects breathing either air or a mixture containing 30% N_2O / 20% O_2 / 50% N_2. Both breathing gases were humidified by passing them through a water bath. The gases were collected in a Douglas bag and the subjects inspired the gases via a low-resistance respiratory valve and mouthpiece (Hans Rudolf, Inc., Kansas City, Missouri). The subjects wore a noseclip throughout the trials. The order of the test conditions (i.e. AIR and N_2O) were randomized. Before each trial the subjects inhaled the appropriate gas mixture during a 15 min rest period in the sitting position. Resting values were recorded during the last 5 min of this rest period. Preliminary trials indicated that 7 min is required for approximately 95% saturation with nitrous oxide.

a) Maximal aerobic exercise: Maximal work rate (Wmax) under control (MAX-AIR) and narcosis (MAX-N_2O) conditions were determined for each subject by performing two $\dot{V}O_{2max}$ tests. $\dot{V}O_{2max}$ was determined on a mechanically braked cycle ergometer (Monark) beginning with a 5

min warm-up at 60 W. Thereafter the load was increased by 30 $W.min^{-1}$ until the subject could not maintain the pedalling rate of 60 rpm. During exercise, a metronome was used to help subjects maintain the cadence. Each trial was terminated after a 10 min recovery period, the first 5 min of which the subjects cycled at 30 W before resting. $\dot{V}O_{2max}$ was identified by a plateau in $\dot{V}O_2$ with increased power output and a respiratory exchange ratio (RER) > 1.10. $\dot{V}O_2$ was compared at 25, 50, 75 and 100% Wmax between the two conditions, relative to the individual subject's maximum work rate.

b) Steady state submaximal exercise: Submaximal $\dot{V}O_2$ under control (SUBMAX-AIR) and narcosis (SUBMAX-N_2O) conditions was determined on a mechanically braked cycle ergometer (Monark, Sweden) beginning with a 5 min warm-up at 30 W followed by 20 min of exercise at 50% of the Wmax obtained during the MAX-AIR trial. During exercise, a metronome was used to help subjects maintain a pedalling rate of 60 rpm. Each trial was terminated after a 5 min recovery period. A 14 ml blood sample was collected during the fifth minute of the warm-up period, the tenth minute of exercise and at the end of the exercise period and analyzed for lactate (see Appendix A). Blood pressure was monitored using the Finapres™ (BOC Ohmeda, Sweden) during exercise. $\dot{V}O_2$ was averaged for each 3 min period before blood samples were collected and compared between the two conditions.

3. Instrumentation:

a) Heart rate (HR . min^{-1}): HR was monitored using an electrocardiograph (Lifepak 8 Cardiac Monitor, Physio-control, Redmond, WA, USA) with recordings made from two pre-gelled electrodes placed laterally on the chest and one on the scapula.

b) Oxygen uptake and carbon dioxide production: Inspired volume was measured with a Alpha Technologies Model VMM110, Turbine Ventilation Module (Laguna Hills, California). Subjects'

expired air was directed to a 9L fluted Plexiglas mixing box, from which a continuous 0.5 $L.min^{-1}$ sample was drawn and analyzed for O_2 and CO_2 content using an Applied Electrochemistry S-3A oxygen analyzer (Ametek Systems, Pennsylvania, USA) and a Statham Godart Capnograph (Godart-Statham B.V., Bilthoven, Holland), respectively. Prior to all trials, the analyzers were calibrated with two calibration gases. For the N_2O trials, the calibration gases contained 30% N_2O in combination with O_2, CO_2, and N_2 prior to the N_2O trial as it is known that nitrous oxide affects both the absorption of infrared by CO_2 and the detection of O_2 by the analyzers (Mekjavic, 1979; Bhavani-Shankar *et al.*, 1992).

c) Core temperature (T_{es}, ^oC): A YSI 702 esophageal thermistor probe (Yellow Springs Instruments, Ohio, USA) was inserted to the level of the right atrium. The insertion length was determined from each subject's sitting height (Mekjavic and Rempel, 1990).

d) Cutaneous blood flow (Perfusion units, PU): Blood perfusion through skin microvessels (to a tissue depth of 1.5 mm) was measured using a Laser Doppler Perfusion Monitor (Periflux PF3, Perimed, Jarfalla, Sweden), set in the wide band range with a 12 kHz upper frequency limit. The laser probe was attached to the right forehead with an adhesive ring.

e) Heat flux ($\dot{Q}$, $W.m.^{-2}$) and skin temperature (T_{sk}, ^oC): Heat flux from the skin surface was measured from transducers (Concept Engineering, Old Saybrook, Connecticutt) placed on four sites (arm, chest, thigh, calf). Thermistors embedded in the transducers allowed simultaneous measurement of skin temperature.

f) Sweating rate (Esw, $g \cdot m^{-2} \cdot min^{-1}$): Esw was measured with a ventilated capsule placed on the forehead. The differences in water vapour density and temperature between the air entering and exiting the capsule (flow rate = 0.5 $L.min^{-1}$) was used to estimated the rate of sweat secretion (see Appendix B).

g) Mean Arterial Pressure (MAP): Blood pressure was measured during the SUBMAX trials, using the 2300 Finapres™ (BOC Ohmeda, Sweden). With the arm supported at heart level, the finger cuff, containing a photoplethysmographic volume transducer, was positioned over the middle phalanx of the middle finger to track finger artery volume changes. MAP was calculated by adding one-third of the difference between the systolic and diastolic pressure to the diastolic pressure.

h) Blood sample collection for lactate: Blood samples were collected from teflon catheters (Angio-Set® 22 ga 3/4 in; Becton Dickinson, Sandy, Utah, USA) inserted into the median cubital vein. The catheters were connected to a one-way stopcock and filled with heparinized saline to facilitate blood sampling (Williams *et al.*, 1992). Subjects remained in a sitting position for at least 10 min to recover from venipuncture before beginning the resting portion of the exercise protocol. Catheter dead space was cleared and the samples collected anaerobically into Vacutainers containing EDTA (Becton Dickinson, Rutherford, NJ, USA). Subjects were asked to refrain from ingesting nicotine or caffeine for 12 hours prior to testing to avoid anomalous plasma catecholamine values.

i)Data acquisition: All data were recorded and stored at 10s intervals using an HP 3497A Data Aquisition System (Hewlett Packard, Anover, Massachussetts), controlled by a microcomputer (Macintosh II, Apple Cupertino, California) with LabView software (National Intruments, Austin, Texas).

4. Data Analysis:

The differences in $\dot{V}O_{2max}$, RER and F_EO_2 at Wmax between the MAX-AIR and N2O trials were tested for significance using paired t-tests. The difference in SUBMAX-$\dot{V}O_2$ between the two conditions was determined using a two-way ANOVA with repeated measures design. In

addition, the final 3 min of the exercise during the SUBMAX trials were averaged for each measured variable and the means analyzed for significance using paired t-tests. The differences in thermal responses during the MAX trials were tested for significance using paired t-tests at rest, 25, 50, 75 and 100% Wmax and during the SUBMAX trials for the mean of the three minutes during blood sample collection. In general, the blood samples were taken within 1 min therefore the 3 min period includes the minute prior to and immediately after collection. The change in core temperature (ΔTes) was defined as the difference between Tes during exercise and Tes at rest. The sweating threshold was defined as the ΔTes at which an increase in capsule humidity was first observed. Results are reported as mean $\pm$ standard error.

RESULTS

All subjects experienced some degree of light-headedness and euphoria associated with narcosis. In general, subjects had no difficulty maintaining the cadence with limited extraneous body movement. Some subjects reported that they felt less fatigued during the N_2O trial and though physically comfortable enough to continue, were unable to make their legs cooperate. One subject was omitted from thermal measurements (N = 8) during the MAX trials and from $\dot{V}O_2$, $\dot{V}CO_2$ and RER measurements (N = 8) during the SUBMAX trials due to equipment malfunction. As a result of heat flux and skin temperature sensor malfunction, mean $\dot{Q}$ and ΔTsk values were determined from chest and calf sensors (N = 8) for the MAX trials however, from all four sensors (N= 6) for the SUBMAX trials. Blood pressure readings during the SUBMAX trials could not be obtained from subjects AL and TM due to poor finger circulation thus these subjects were excluded from MAP calculations (N = 7).

Maximal aerobic exercise:

1. Oxygen uptake

Each subject attained the same Wmax in both the N_2O and the AIR trials. The $\dot{V}O_2$-relative work rate relation, from unloaded pedalling to Wmax, was similar during N_2O and AIR prior to subjects approaching $\dot{V}O_{2max}$ (Fig. 1). $\dot{V}O_{2max}$ was significantly higher (P = 0.008) at 58.9 ± 3.1 $ml.kg^{-1}.min^{-1}$ when subjects breathed 30% N_2O compared to 55.0 ± 2.4 $ml.kg.^{-1}$ min^{-1} when air was inhaled (Table 2). RER was lower during narcosis (1.15 ± 0.02 compared to 1.25 ± 0.03 at 100% Wmax), but this difference did not become significant until subjects were exercising at greater than 75% Wmax (P = 0.004 at 100% Wmax; Fig. 2). $\dot{V}CO_2$ showed similar increases with work during both trials (Fig. 3). F_EO_2 was significantly lower during the N_2O trial at 100%Wmax (0.169 ± 0.001 compared 0.172 ± 0.002 for the AIR trial, P = 0.006, Fig. 4). No significant differences were observed in F_ECO_2, $\dot{V}_I$ and HR (Table 2).

2. Thermal response

No significant differences were observed between the two trials in the response of Esw with change in core temperature (ΔTes; Fig. 5), nor in the esophageal threshold temperature for the onset of sweating (AIR: ΔTes = 0.16 ± 0.07 °C, N_2O: ΔTes = 0.20 ± 0.03 °C). During both trials, heat flux from the surface of the skin increased with skin temperature (Fig. 6). Cutaneous blood flow increased with exercise intensity during both conditions (Fig. 7). No significant differences were observed in either SkBP or $\dot{Q}$ between the two trials.

Submaximal aerobic exercise:

1. Oxygen uptake

There was no significant difference in $\dot{V}O_2$ during steady-state exercise at 50% Wmax when subjects inhaled air or the 30% nitrous oxide mixture (Fig. 8). Table 3 reports mean values for the final 3 min of exercise. $\dot{V}O_2$ was 32.9 ± 1.0 ml.kg.$^{-1}$min.$^{-1}$ during the AIR trial compared to 32.4 ± 1.6 ml.kg.$^{-1}$min.$^{-1}$ during the N_2O trial. No significant differences were observed in HR, $\dot{V}_I$, RER, F_EO_2 (Fig. 9), and F_ECO_2. $\dot{V}CO_2$ was similar during both SUBMAX trials (Fig. 10). Plasma lactate values were not significantly different between the times at which samples were collected nor between the two conditions (Fig. 11). The observed reduction in mean arterial pressure (MAP) during the N_2O trial was not significant when compared to the AIR trial (Fig. 12).

2. Thermal response

There were no significant differences in the responses of Esw (Fig. 13), $\dot{Q}$ with ΔTsk (Fig. 14), SkBP (Fig. 15), ΔTes, and the onset of sweating. Excessively low heat flux and skin temperature readings were obtained for 3 subjects from at least one of the sensors. This was

attributed to poor sensor attachment during profuse sweating and to a malfunction of the calf sensor therefore these subjects were excluded from mean $\dot{Q}$ and ΔTsk determinations. AIR-ΔTes at the end of the 20 min exercise period was 0.51 ± 0.11 °C while N_2O-ΔTes was 0.43 ± 0.10 °C. The onset of sweating occurred at a ΔTes of 0.07 ± 0.13 and -0.02 ± 0.14 °C during the AIR and N_2O trials, respectively.

DISCUSSION

The purpose of this study was to investigate whether narcosis, induced by nitrous oxide, alters oxygen utilization during muscular work to elucidate the mechanism underlying the narcosis-induced decrease in $\dot{V}O_2$ observed during cold-water immersion. The results indicate that there is no difference in $\dot{V}O_2$ during exercise between the AIR and N_2O conditions until subjects reach maximal work intensity. Heat loss mechanisms were unaffected by narcosis, as reflected in Esw, SkBP and $\dot{Q}$, and therefore thermal balance was not compromised. Thus, this study confirms that the narcosis-induced decrease in $\dot{V}O_2$ during cold water immersion is not due to reduced oxygen utilization by the cells.

1. Aerobic performance

The present study observed no difference in $\dot{V}O_2$ between the AIR and N_2O trials when subjects were exercising at 50% of their individually determined Wmax, whereas Bradley and Dickson Jr. (1976) observed a significant decrease in $\dot{V}O_2$ during steady-state exercise at a moderate work load when subjects were breathing a 30% N_2O mixture. However, when Bradley and Dickson Jr. doubled the work load, $\dot{V}O_2$ was not significantly different between the two conditions. During the MAX trials of this study, $\dot{V}O_2$ was similar during both trials until subjects approached 100% Wmax, resulting in a 7 % increase in $\dot{V}O_{2max}$ during narcosis. The lack of any affect of N_2O on steady state $\dot{V}O_2$ during the SUBMAX tests and prior to 100% Wmax during the MAX tests suggests that narcosis does not affect $\dot{V}O_2$ at less than maximal power output. In the study of Bradley and Dickson Jr., the similarity in $\dot{V}O_2$ between the air and narcosis conditions during heavy exercise could be attributed to the increase in ventilation when subjects inhaled 30% N_2O, whereas during moderate exercise no significant difference in ventilation was observed. Ventilation was not significantly affected by narcosis in this study, at any given work rate.

The effects of inhaling analgesic levels of volatile anesthetics on aerobic exercise are relatively unknown in the literature, thus it is necessary to turn to hyperbaric studies to compare the results of this study. Fagraeus *et al.* (1973) observed a 9% increase in $\dot{V}O_{2max}$ at 1.4 ATA and a 5 to 6% increase at 2.0 and 3.0 ATA which they attributed to improved oxygen delivery with increased arterial PO_2 (P_aO_2) as a result of raising the environmental air pressure. The tendency for $\dot{V}O_{2max}$ to decrease toward control values when the pressure was increased above 1.4 ATA, was explained by ventilatory impairment and CO_2 retention offsetting the beneficial effects of hyperoxia (Fagraeus, 1974). However, it could not be excluded that N_2 molecules may have interfered with the diffusion of O_2 (Fagraeus, 1974). This study demonstrates that narcosis does not affect $\dot{V}O_2$ at a given work rate and does not decrease $\dot{V}O_{2max}$ therefore it is likely that hyperbaric N_2 had no affect on the drop in $\dot{V}O_{2max}$ observed by Fagraeus *et al.* (1973).

The increased $\dot{V}O_{2max}$ observed during narcosis in the present study cannot be attributed to hyperoxia since the inspired PO_2 levels were the same in both conditions. Cook (1970) avoided the effects of hyperoxia at 3.0 ATA by maintaining normoxia during treadmill exercise to exhaustion. He observed a decrease in $\dot{V}O_{2max}$ and work capacity to 68 and 58% of the control value, respectively. This decrease was accompanied by a reduction in ventilation to 38% of the control value and raised alveolar PCO_2 with increased metabolic acidosis which were again, attributed to ventilatory insufficiency at pressure. Because of this large drop in work capacity, it is questionable whether subjects stopped exercising due to attainment of $\dot{V}O_{2max}$ or whether other mechanisms were limiting their ability to perform muscular work.

Eiken *et al.* (1987) suggested that raised PN_2 and/or pressure *per se* may interfere with neural input to exercising muscles or impair the excitation-contraction coupling mechanism based on observations of reduced peak torque during maximal unilateral knee extensions at 6.0 ATA. They also observed that at increased pressure maximal force was reduced to a greater extent in subjects with a greater percentage of fast twitch (FT) fibres, suggesting that there might be selective

interference of neural input to these fibres. These findings are supported by studies demonstrating an increase in the activation threshold of motor neurons exposed to non-volatile (Thesleff, 1956; Somjen, 1967) and volatile anesthetics (Somjen, 1967; de Jong *et al.*, 1969) suggesting that narcosis *per se* could be partially responsible for interfering with neural input to working muscles. An overall increase in motor neuron excitation threshold could prevent sufficient fast twitch fibre activation and reduce the work capacity of the exercising muscles. If N_2O affects neural input to the exercising muscles and alters the recruitment pattern this could lead to activation of accessory muscle groups working at a mechanical disadvantage to maintain force output. As the load is increased during a progressive cycle test there is increasing activation of hip extensors, namely the *gluteus maximus*, as the other working muscles fatigue (Green and Patla, 1992). The increased $\dot{V}O_{2max}$ observed when subjects inhaled N_2O may be attributed to increased involvement of accessory muscles at Wmax to maintain the force output.

Regardless of whether incremental exercise is performed in a hyperbaric environment or while a volatile anesthetic is inhaled, an increase in $\dot{V}O_{2max}$ must be explainable in terms of either an increase in blood flow through the working leg muscles or a greater arteriovenous O_2 difference, $(a\text{-}v)O_2$. Since neither HR nor MAP were significantly higher during the SUBMAX-N_2O trial compared to the AIR trial, it seems unlikely that increased cardiac output was responsible for the higher $\dot{V}O_{2max}$ during narcosis, assuming MAP would respond to narcosis in a similar manner during maximal exercise. Because changes in diastolic pressure are minimal during incremental exercise, MAP will also change very little, thus lack of an obvious increase in MAP does not necessarily exclude the possibility of an increase in stroke volume and thus increased cardiac output. Systolic pressure differences between the two conditions would be a better indicator of whether there was a change in cardiac output. The mean systolic pressure for the last 3 min of exercise during the SUBMAX trials was not significantly different (AIR: 162 ± 11 mmHg; N_2O: 155 ± 8 mmHg). It was assumed that the errors in the Finapres™ measurements during the SUBMAX trials were small and consistent. Blood pressure was not measured during maximal

exercise because it has been demonstrated that the Finapres™ overestimates systolic pressure during strenuous exercise (Wesseling, 1989). It cannot be excluded that the response of blood pressure to narcosis during incremental exercise is different from the response to narcosis during submaximal exercise. The tendency for a blunted exercise response in HR observed during the MAX-N_2O trial suggests that the effects of N_2O on sympathetic outflow were similar for both the MAX and SUBMAX-N_2O trials. If the increase in $\dot{V}O_{2max}$ was not due to an increase in cardiac output, an increase in the (a-v)O_2 difference must be considered.

Increased oxygen extraction by the tissues would increase the (a-v)O_2 difference. The observation that $\dot{V}O_{2max}$ was higher during the MAX-N_2O trial may appear contrary to the reduced $\dot{V}O_{2max}$ observed by Cook (1970) where the ambient pressure had an influence on ventilatory capacity. However, given the 62% drop in ventilation, one would expect Cook (1970) to have observed a greater drop in $\dot{V}O_{2max}$. This discrepancy can be explained by the 15% drop in expired oxygen which accompanied the drop in $\dot{V}O_{2max}$, suggesting that at 3.0 ATA the cells utilized oxygen more efficiently. The mechanism by which the efficiency of oxygen utilization would improve during alveolar hypoxia under these conditions is unclear. One possibility is that oxygen delivery is improved by an increase in blood flow to the contracting muscles (Hogan and Welch, 1986) however, analgesic levels of N_2O are known to increase alpha-adrenergic stimulation, resulting in increased peripheral vascular resistance (Eisele, 1985). Nevertheless, the results of the present study are supported by studies which have shown that anesthetic levels of N_2O shift the oxyhemoglobin dissociation curve to the right (Smith *et al.*, 1970; Kambam, *et al.*, 1983), suggesting that the extraction of oxygen is improved as the affinity of hemoglobin (Hb) for oxygen is reduced (Lanza *et al.*, 1988). N_2O may reduce the affinity of Hb for oxygen by interfering with the O_2 - Fe bond in Hb (Lanza *et al.* 1988). Since the higher $\dot{V}O_2$ during narcosis relative to normal air is not apparent until the subject approaches Wmax, it seems reasonable to suggest that when 30% N_2O is inhaled there may be an increased sensitivity of Hb

to the drop in pH associated with the accumulation of lactate at higher workloads, causing Hb to release more oxygen to the tissues.

Though RER was lower during both the MAX and SUBMAX-N_2O trials compared to corresponding AIR trials, the decrease was not significant until subjects approached $\dot{V}O_{2max}$ during the MAX-N_2O trial. This suggests either a greater reliance on fatty acid metabolism to meet energy requirements or a reduction in plasma lactate. During maximal exercise, accumulation of lactate causes excess CO_2 production, making it difficult to estimate the relative contribution of carbohydrate and fatty acid metabolism (Jones *et al.*, 1980). In addition, accumulation of lactate and the corresponding decrease in muscle pH are strong inhibitors of plasma FFA utilization (Bjorntorp, 1965; Fredholm, 1971) and are also likely regulators of muscle triglyceride utilization (Gorski, 1992) so it is unlikely that increased lipolysis occurred. Inferences regarding plasma lactate concentrations during the MAX trials cannot be made based on the results of the SUBMAX trials where, given the similar plasma lactate concentrations between unloaded and 50% Wmax exercise, the intensity of exercise was not great enough to significantly mobilize anaerobic energy stores. However, it cannot be excluded that the lactate concentration may have been lower and the muscle pH higher during the MAX-N_2O trial or that N_2O affected membrane transport of fatty acids. According to Lewis and Haller (1989), 10% more oxygen is required to synthesize a mole of ATP from free fatty acids than from glycogen. An increase in mobilization of muscle triglycerides, usually occurring in trained atheletes (Hurley *et al.*, 1986) could account for an increase in $\dot{V}O_2$ however, for this to occur at maximal work rates during narcosis, the cells of the working muscles would have to increase permeability to fatty acids and extract more oxygen than when subjects are performing the same exercise while breathing air. Anesthetic agents such as N_2O may alter membrane permeability by expanding the lipid membrane and altering the shape of the transport channels (Miller *et al.*, 1973). There was no change in $\dot{V}CO_2$ production between the two conditions during either the MAX or SUBMAX tests suggesting that carbohydrate metabolism was unaffected by narcosis. Increased activation of

accessory muscles at Wmax during narcosis may account for a greater fuel and O_2 requirement to maintain the work load however, it is unclear why there would be an increase in fatty acid metabolism rather than carbohydrate metabolism during maximal exercise.

Despite a higher $\dot{V}O_{2max}$ during narcosis, the similarity between the $\dot{V}O_2$-relative work rate relations during both incremental and steady-state exercise indicates that there was no difference in the amount of oxygen utilized to perform a given submaximal work rate when the subjects were breathing either 30% N_2O or air. The lack of any significant changes in the heat balance components between the two conditions would support the observation that there was no difference in the relative intensity of work performed however, exercise at Wmax may not have been long enough for any changes to become apparent.

2. Thermal considerations

This study has shown that the decrease in $\dot{V}O_2$ during narcosis and cold-water immersion (Mekjavic and Sundberg, 1992; Passias *et al.*, 1992) cannot be attributed to a decrease in utilization of oxygen by the muscle cells. Therefore, reduced neural input seems a more likely explanation for the attenuated shivering response. If oxygen extraction is also improved, reduced shivering during narcosis may be attributed to opposing effects of decreased neural input to the muscles and increased oxygen utilization by the muscles to produce an overall reduction in $\dot{V}O_2$. Since the intensity of muscular activity during shivering is comparable to the intensity of muscular activity during submaximal exercise where narcosis did not affect $\dot{V}O_2$, it is unlikely that there would be an increase in oxygen extraction by the shivering muscles. Cheung and Mekjavic (1994) monitored shivering EMG activity, demonstrating both a reduction in neural input to the muscles during cold water immersion and a corresponding reduction in shivering $\dot{V}O_2$ in response to N_2O-induced narcosis. In the present study, exercise $\dot{V}O_2$ was not significantly different for a given submaximal work rate between the two conditions, suggesting that contrary to the effects of

narcosis on the involuntary muscle activity of shivering, narcosis does not limit performance during repeated voluntary muscle contractions of moderate intensity.

The observation of the present study that the onset of sweating was unaffected during narcosis contradicts previous findings that the sweating threshold is increased when subjects are passively heated during enflurane and isoflurane anesthesia (Lopez *et al.*, 1993; Washington *et al.*, 1993). However, these studies agree with the present study that there is no change in the gain of the response. Thermoregulatory responses are a function of both core and skin temperatures (Benzinger, 1969), thus in subjects that are passively heated by exogenous heat, the thermal afferent drive from peripheral warm sensors to the central thermoregulatory centre is greater than in exercising subjects where thermal afferent drive is mainly from core sensors responding to endogenous heat and/or to non-thermal factors. Because the changes in skin temperature are small during exercise, the peripheral afferent input is attenuated relative to when the skin is directly heated. It also cannot be excluded that the level of anesthesia affects the sweating threshold. Whereas this study was conducted with an analgesic level of anesthetic gas, the studies which observed an elevated threshold for sweating (Lopez *et al.*, 1993; Washington *et al.*, 1993) were conducted on subjects under general anesthesia.

In conclusion, mild inert-gas narcosis does not affect work performance or heat dissipation mechanisms during aerobic exercise. This finding confirms that the narcosis-induced reduction in $\dot{V}O_2$ observed during cold-water immersion studies is not related to a decrease in O_2 utilization by the cell mitochondria. This finding is also of importance in the controversy surrounding the use of N_2O by women to alleviate pain during intense labor (Eger, 1985). There is a concern that hypoxia may result from inhalation of N_2O, endangering the mother and fetus. It has been demonstrated that arterial haemoglobin O_2 saturation (SaO_2) is significantly reduced when N_2O is administered with narcotics such as pethidine whereas SaO_2 is unaffected when N_2O is administered alone (Zelcer *et al.*, 1989). The results of the present study confirm that when a

normoxic mixture of 30% N_2O is inhaled, hypoxia does not occur during exercise of the intensity experienced with normal labor (Katz *et al.*, 1990) and given the high levels of oxygen consumption during maximal exercise, hypoxia is also unlikely to occur during heavy labor.

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APPENDICES

APPENDIX A: Lactate analysis

Immediately following collection, the blood samples were placed on ice and centrifuged at 0 - 4 ^{o}C for 15 min at approximately 2500 x g. 2 ml of the supernatent was removed from each sample and added to 4 ml cold 8% perchloric acid (PCA). This mixture was vortexed for approximately 30 sec, allowed to sit on ice for 5 min to ensure deproteinization was complete before centrifuging at approximately 1500 x g for another 10 min. The plasma was transfered into test-tubes, capped and stored at -80 ^{o}C for 4 - 6 months before analysis.

In the assay used to measure plasma lactate, the catalytic action of lactate dehydrogenase (LD) allows lactate to be measured in terms of the generation of NADH spectrophoto-metrically at 340 nm (Bergmeyer, 1984). After the samples were allowed to thaw at room temperature, they were recentrifuged to obtain a clear protein-free solution, then placed at -20 ^{o}C until they could be analyzed the next day. Lactate analysis kits (Sigma Diagnostics, St. Louis, MO, USA) were used with minor changes to the procedure. Rather than comparing sample absorbances to a blank, initial absorbance readings (Lambda 2 UV/VIS Spectrometer, Perkin-Elmer Corp., Norwalk, CT, USA) were made of the 2 replicates of each sample before addition of LD. The enzyme was then added and the samples allowed to sit in a water bath at 37 ^{o}C until completion of the reaction, identified when the drift of the absorbance reading of the blank was equal to the drift of the sample. Absorbance was calculated as the difference between the final absorbance and the pre-enzyme absorbance minus the drift of the blank. The following equation was used to calculate plasma lactate concentration (mmol.L^{-1}):

$$[\text{lactate}] = (\Delta A_{340} \times V_T) / (6.22 \times V_S \times d)$$

where : ΔA_{340} = Final maximum absorbance at 340 nm

V_T = Total reaction volume in cuvet (1.5 ml)

6.22 = Millimolar absorptivity of NADH at 340 nm

V_S = Volume of plasma sample in cuvet (0.01665 ml)

d = Length of lightpath (1 cm)

APPENDIX B: Calculation of Sweating Rate (Esw)

Esw was measured using resistance hygrometry by passing dry compressed air (flow rate = 0.5 L/min) into an air-tight capsule placed on the left forehead and measuring the temperature and humidity of the outflowing air. The following equation was used to calculate Esw:

$$Esw = \{ \{ [(RH_o/100) \times 5598.6 \times e^{(0.0565 \times To)}] - [(RH_i/100) \times 5598.6 \times e^{(0.0565 \times Ti)}] \} \times V_S \} / A$$

where: RH = relative humidity of inflowing (i) or outflowing (o) air

T = air temperature

A = surface area under capsule

V_S = flow rate of air entering capsule

Because the inflowing air did not contain any moisture (i.e. $RH_i = 0$) the equation reduced to:

$$Esw = \{ [(RH_o/100) \times 5598.6 \times e^{(0.0565 \times To)}] \times V_S \} / A$$

TABLES AND FIGURES

Subject	Sex	Age (yr)	Height (cm)	Weight (kg)
AL	M	27	183	72
CE	M	30	193	85
GA	M	30	181	81
IM	M	36	178	72
MW	M	40	186	86
PZ	M	24	183	91
RD	M	23	183	63
SR	M	28	170	67
TM	M	39	183	72
TP	M	26	178	84
Mean ± SE		30.6 ± 3.2	183.1 ± 1.4	78.4 ± 2.9

TABLE 1: Anthropometric data. Subject GA included in MAX trials only. Subject SR included in SUBMAX trials only.

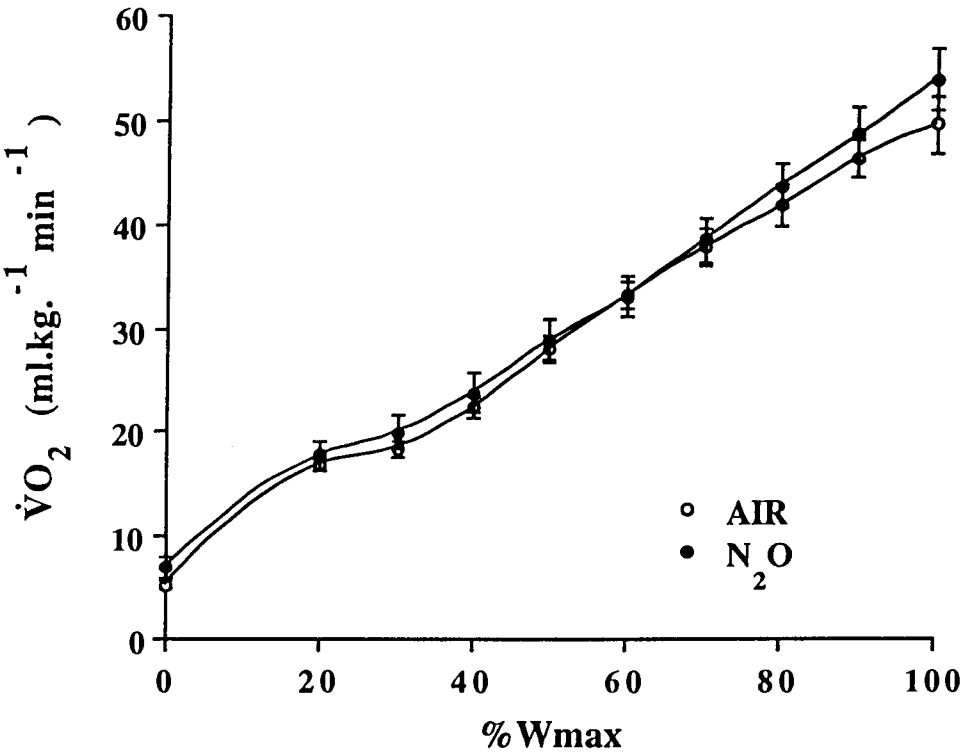

FIGURE 1: Oxygen uptake during incremental exercise while inhaling air or 30% nitrous oxide (N = 9; mean ± SE). P = 0.008 at $\dot{V}O_2$ max.

Subject	Trial	HR (min^{-1})	$\dot{V}_I$ ($L.min^{-1}$)	RER	F_EO_2	F_ECO_2	$\dot{V}O_2max$ ($ml.kg.^{-1}$ min^{-1})
AL	AIR	188	112.6	1.19	0.168	0.047	64.9
	N_2O	179	122.7	1.20	0.168	0.047	70.5
CE	AIR	218	116.4	1.23	0.175	0.046	53.4
	N_2O	181	113.0	1.15	0.169	0.045	57.4
GA	AIR	202	110.0	1.30	0.173	0.044	48.8
	N_2O	199	91.4	1.22	0.171	0.045	47.3
IM	AIR	213	123.0	1.18	0.179	0.035	51.9
	N_2O	172	102.3	1.13	0.173	0.039	53.2
MW	AIR	171	121.4	1.39	0.179	0.039	45.6
	N_2O	173	109.9	1.15	0.176	0.038	46.2
PZ	AIR	199	114.4	1.21	0.167	0.049	56.6
	N_2O	218	124.3	1.06	0.168	0.043	60.1
RD	AIR	186	78.6	1.38	0.173	0.044	56.3
	N_2O	189	106.4	1.20	0.171	0.044	65.2
TM	AIR	187	106.1	1.10	0.167	0.046	66.8
	N_2O	181	100.9	1.06	0.163	0.048	72.4
TP	AIR	182	112.0	1.27	0.171	0.046	50.7
	N_2O	220	102.6	1.19	0.164	0.052	57.6
Mean	AIR	194	110.5	1.25	0.172	0.044	55.0
	N_2O	190	108.2	1.15*	0.169*	0.045	58.9*
SE	AIR	5	4.4	0.03	0.002	0.001	2.4
	N_2O	6	3.5	0.02	0.001	0.001	3.1

TABLE 2: Measurements at Wmax, during the final minute of exercise. *Asterisks indicate N_2O values that are significantly different from AIR values ($P < 0.05$).

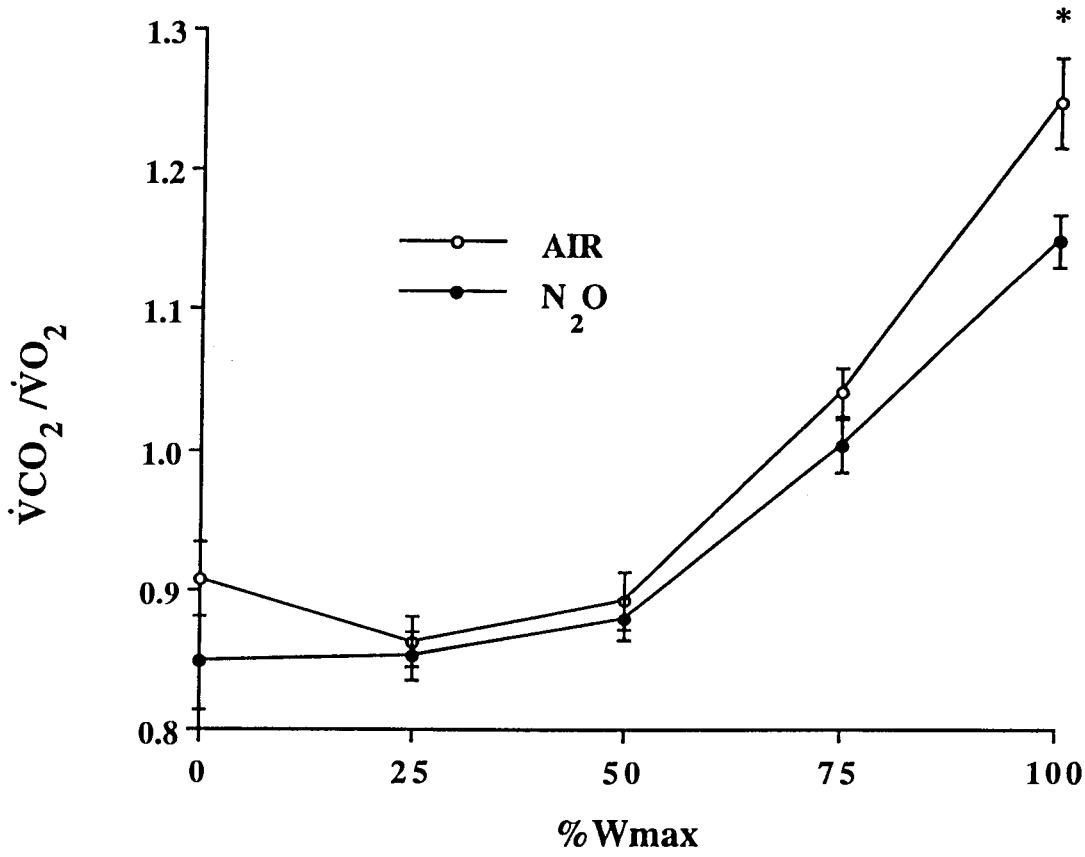

FIGURE 2: Respiratory exchange ratio during incremental exercise while inhaling air or 30% nitrous oxide (N = 9; mean ± SE). *P = 0.004

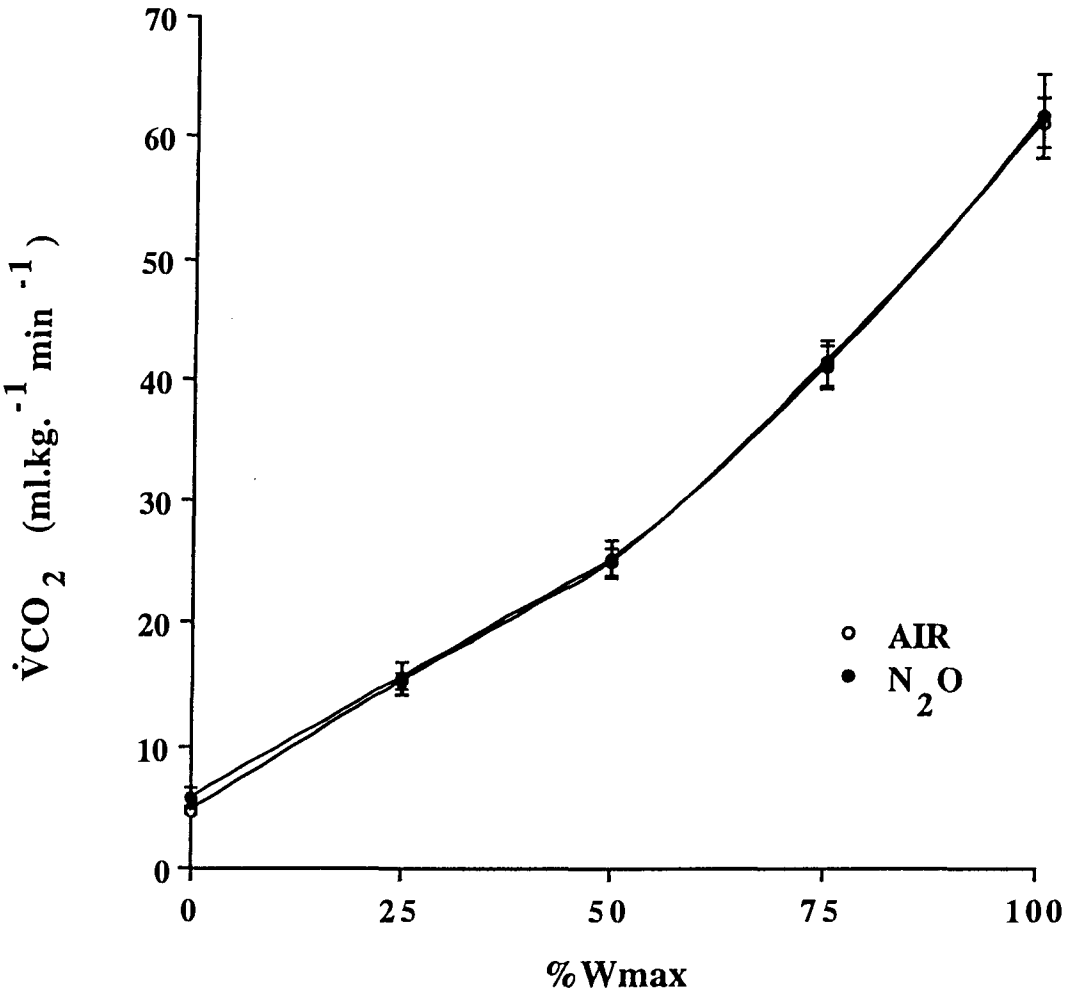

FIGURE 3: Carbon dioxide production during incremental exercise while inhaling air or 30% nitrous oxide (N = 9; mean ± SE).

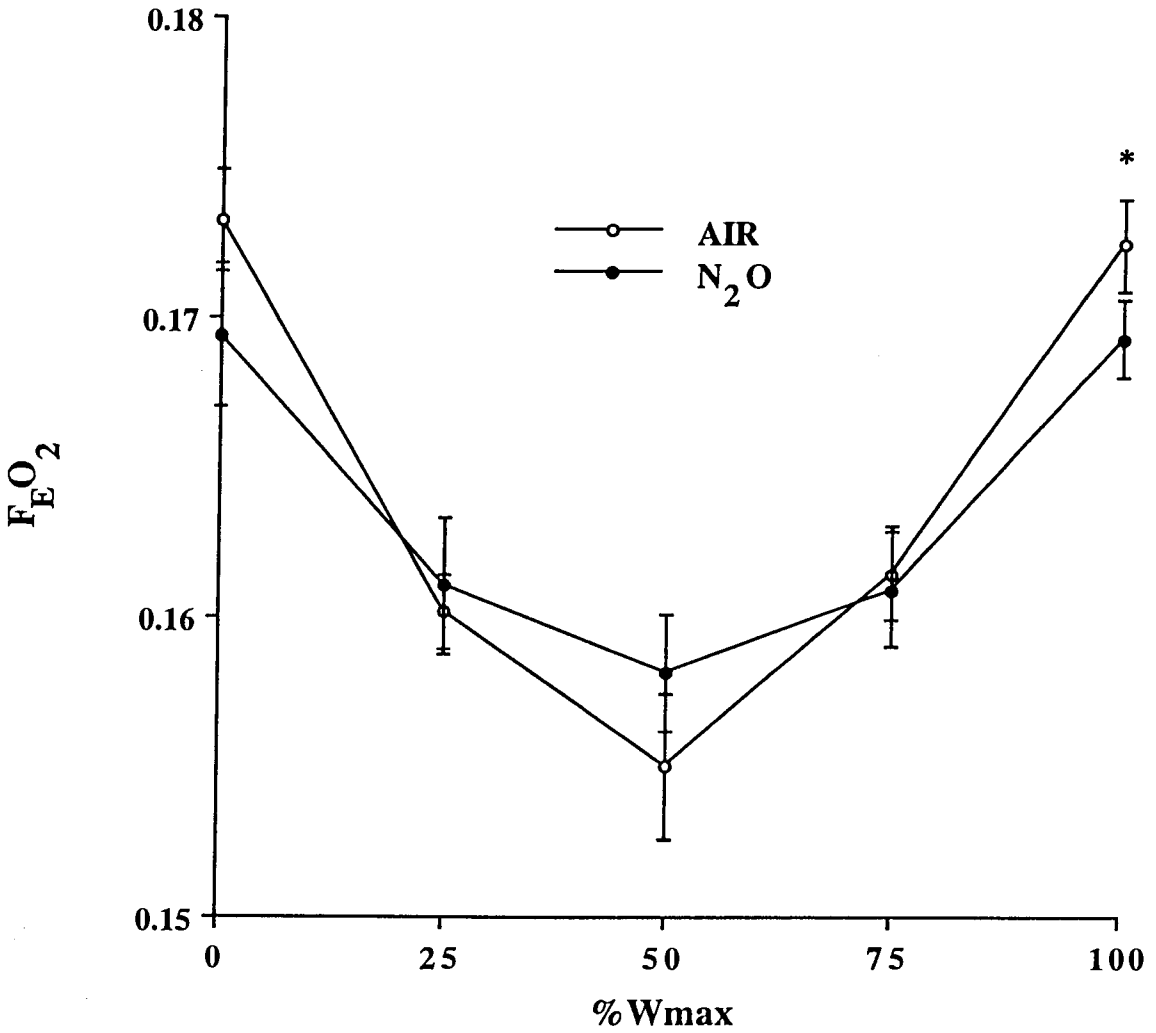

FIGURE 4: Fraction of expired oxygen during incremental exercise while inhaling air or 30% nitrous oxide (N = 9; mean ± SE). *P = 0.006

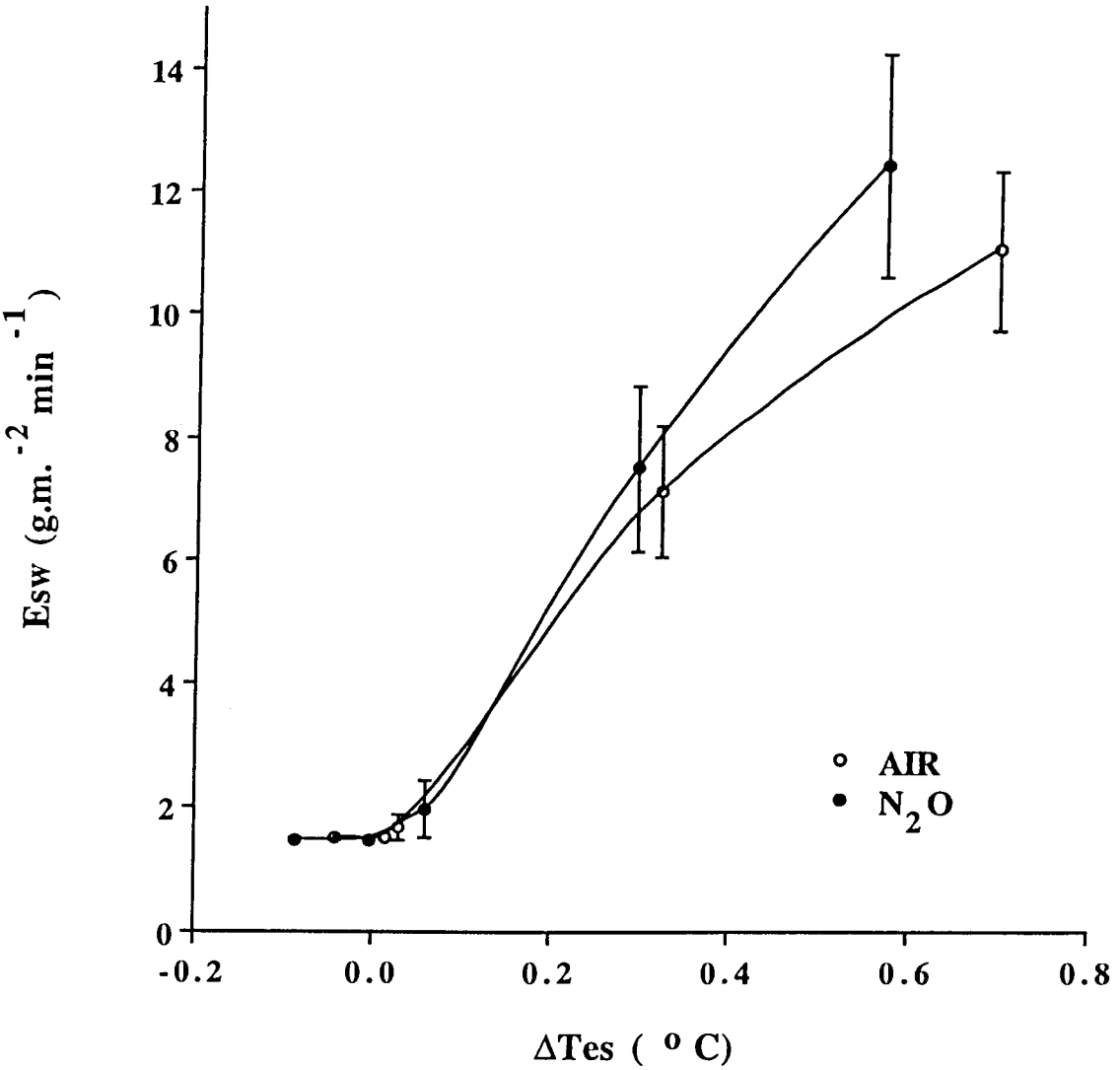

FIGURE 5: Sweating rate with change in core temperature at rest, 25, 50, 75 and 100% Wmax while inhaling air or 30% nitrous oxide (N = 8; mean ± SE).

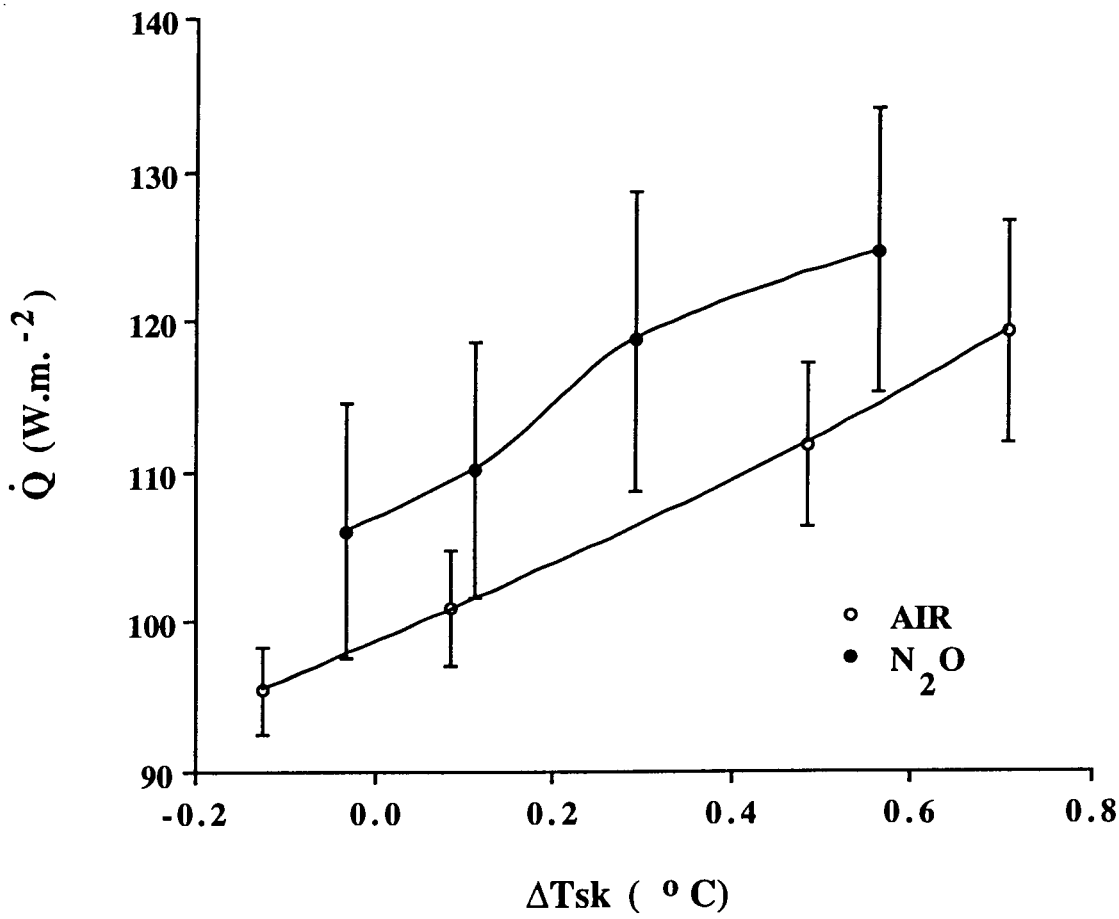

FIGURE 6: Heat flux with change in skin temperature at rest, 25, 50, 75 and 100% Wmax while inhaling air or 30% nitrous oxide (N = 8; mean ± SE).

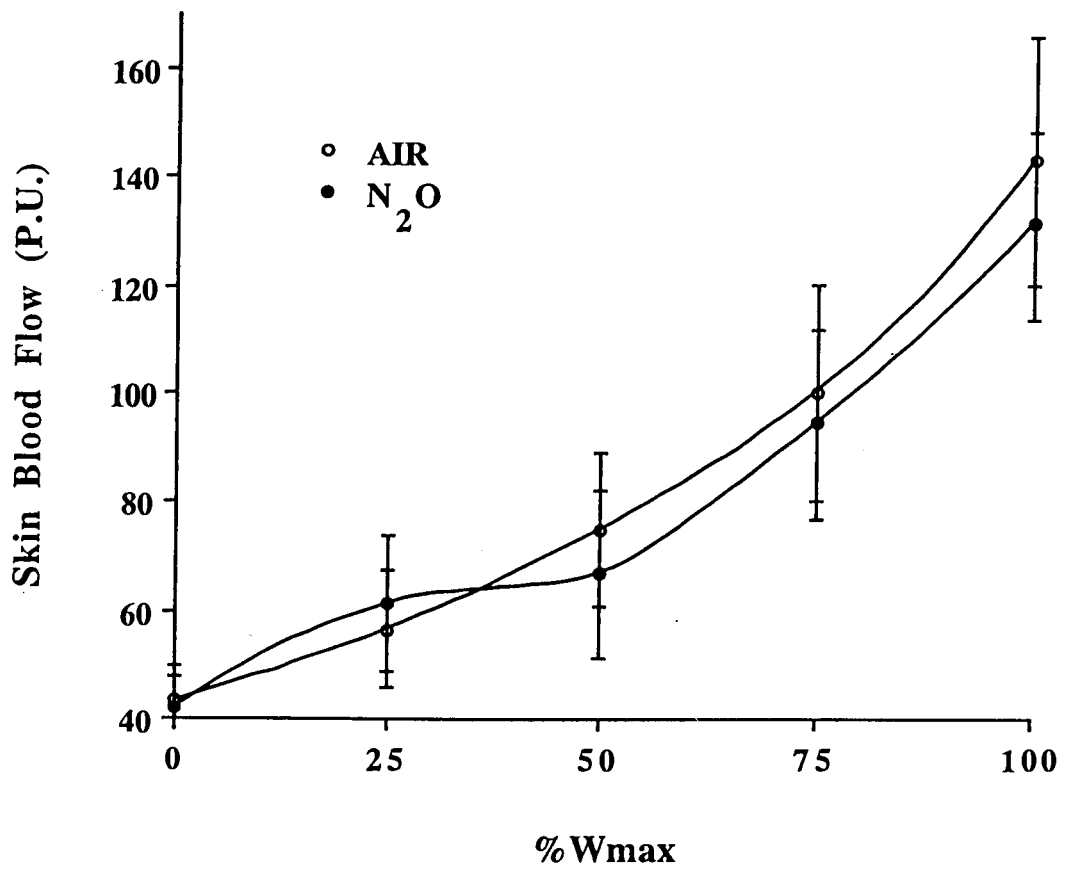

FIGURE 7: Cutaneous blood flow (perfusion units) during incremental exercise while inhaling air or 30% nitrous oxide (N = 9; mean ± SE).

Subject	Trial	HR (min^{-1})	$\dot{V}_I$ ($L.min^{-1}$)	RER	F_EO_2	F_ECO_2	$\dot{V}O_2$ ($ml.kg.^{-1}$ min^{-1})
AL	AIR	150	48.2	0.93	0.157	0.050	37.2
	N_2O	154	56.3	0.93	0.160	0.047	40.8
CE	AIR	137	50.4	0.97	0.161	0.047	30.3
	N_2O	147	57.5	0.94	0.161	0.046	34.5
IM	AIR	117	53.0	0.89	0.169	0.037	31.7
	N_2O	109	49.4	0.90	0.166	0.040	31.6
MW	AIR	137	54.7	0.95	0.165	0.042	28.2
	N_2O	137	47.1	0.97	0.162	0.046	26.0
PZ	AIR	161	65.1	0.98	0.163	0.046	31.7
	N_2O	155	66.8	0.90	0.167	0.039	30.3
RD	AIR	167	48.0	1.06	0.163	0.049	34.6
	N_2O	160	48.1	0.97	0.164	0.044	34.5
SR	AIR	150	47.5	0.93	0.161	0.046	34.5
	N_2O	139	46.5	0.95	0.163	0.045	32.3
TM[a]	AIR	142	53.1		0.160		
	N_2O	134	54.4	0.90	0.162	0.044	35.8
TP	AIR	158	59.4	1.03	0.162	0.049	34.7
	N_2O	140	48.6	0.90	0.161	0.044	29.2
Mean	AIR	147	53.3	0.97	0.162	0.046	32.9
	N_2O	142	52.7	0.93	0.163	0.044	32.4
SE	AIR	5	2.0	0.02	0.001	0.001	1.0
	N_2O	5	2.2	0.01	0.001	0.001	1.6

TABLE 3: Mean values for the last 3 minutes of exercise at 50% Wmax. No significant differences were observed between N_2O and AIR values ($P > 0.05$). [a]Invalid $FeCO_2$ measurements, thus excluding subject TM data from calculations requiring this variable.

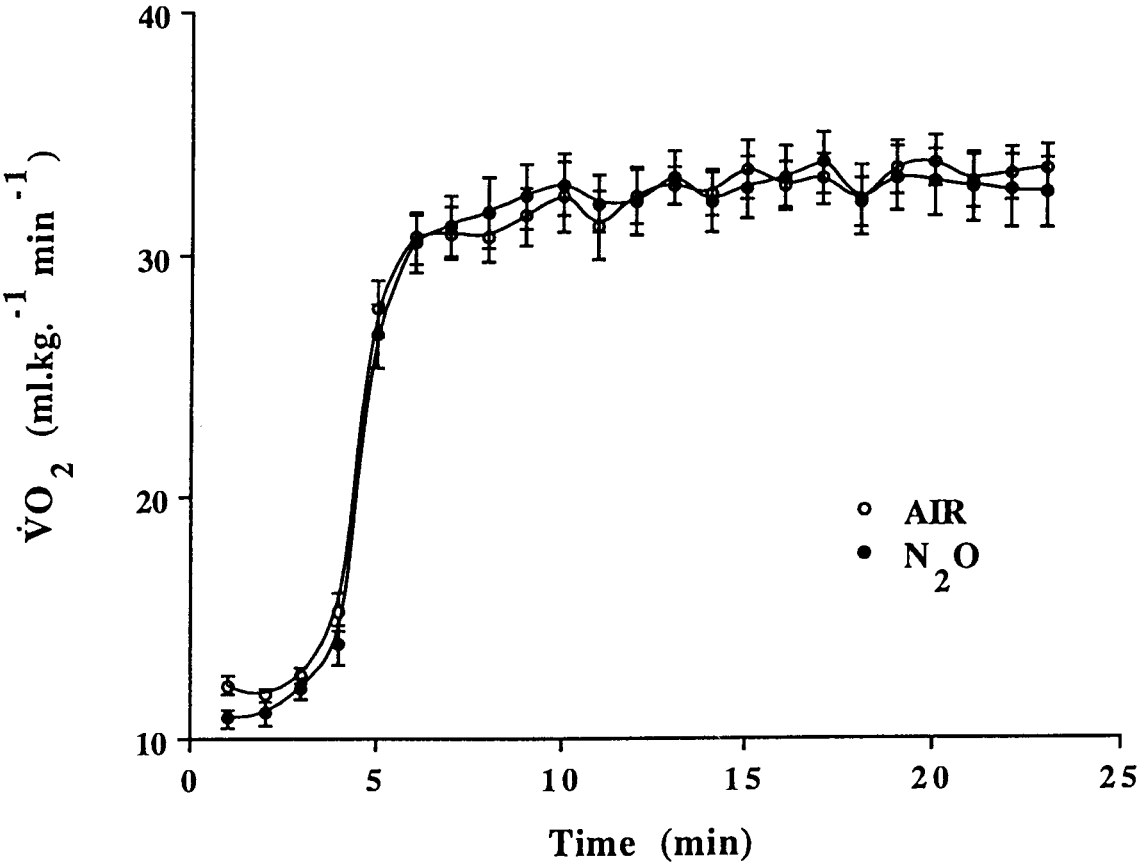

FIGURE 8: Oxygen uptake while breathing air or 30% nitrous oxide during 5 min of unloaded pedalling followed by 20 min at 50% Wmax (N = 8; mean ± SE).

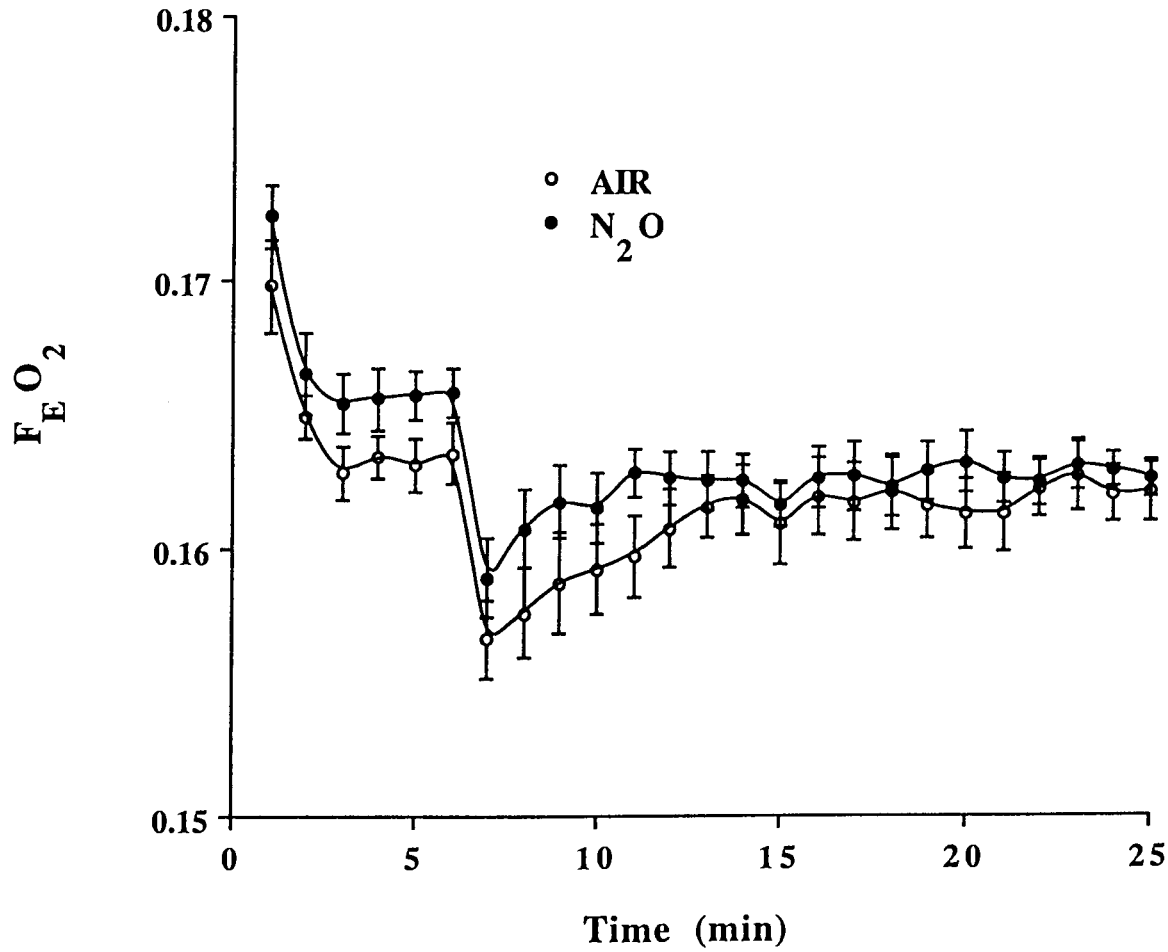

FIGURE 9: Fraction of expired oxygen during 5 min unloaded pedalling and 20 min at 50% Wmax while inhaling air or 30% nitrous oxide (N = 9; mean ± SE).

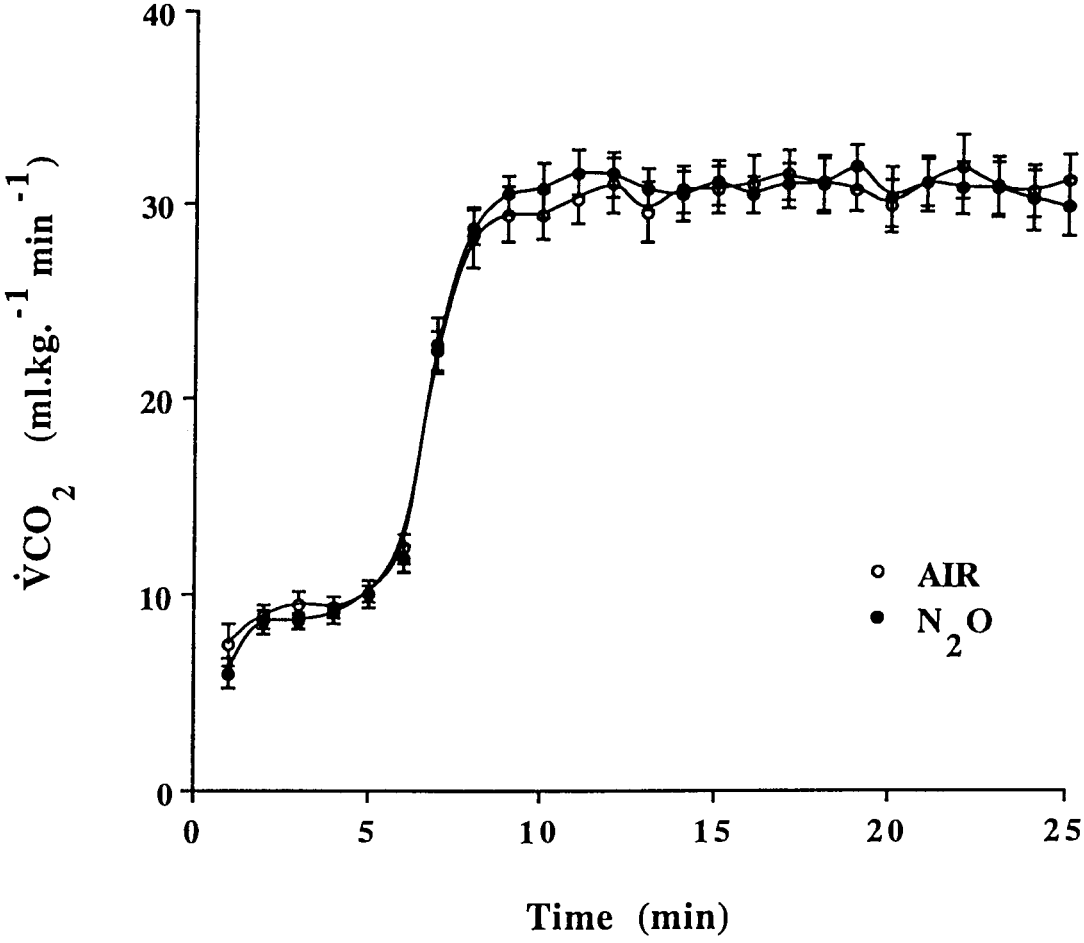

FIGURE 10: Carbon dioxide production while inhaling air or 30% nitrous oxide during 5 min of unloaded pedalling followed by 20 min at 50% Wmax (N = 8; mean ± SE).

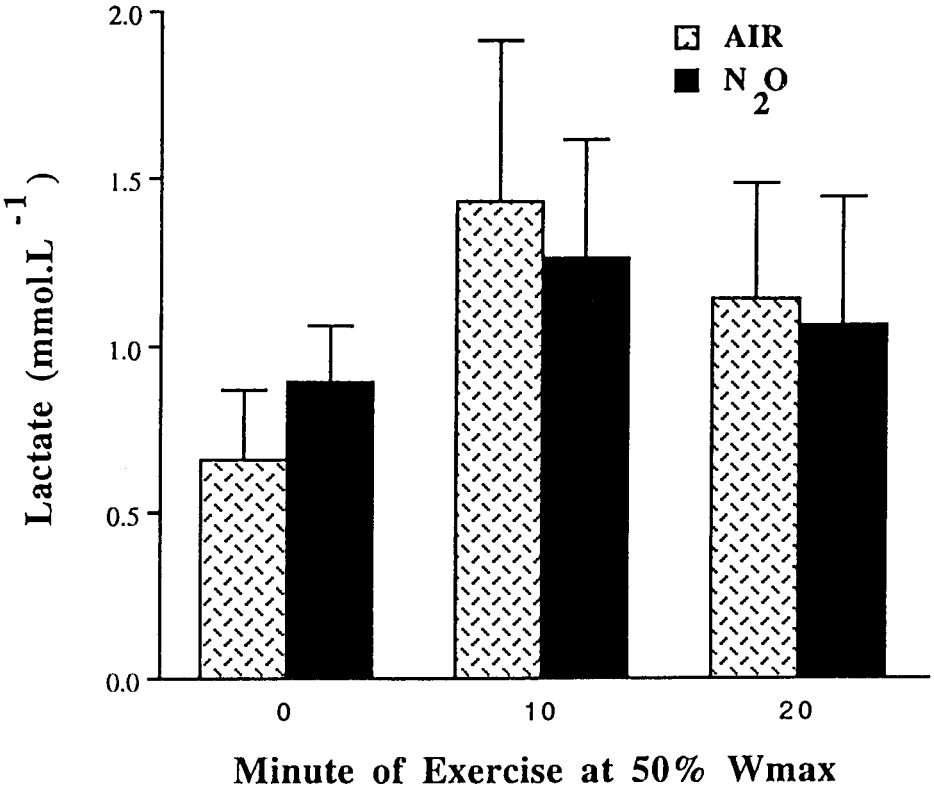

FIGURE 11: Plasma lactate values at minutes 0, 10 and 20 at 50% Wmax while inhaling air or 30% nitrous oxide (N = 9; mean ± SE).

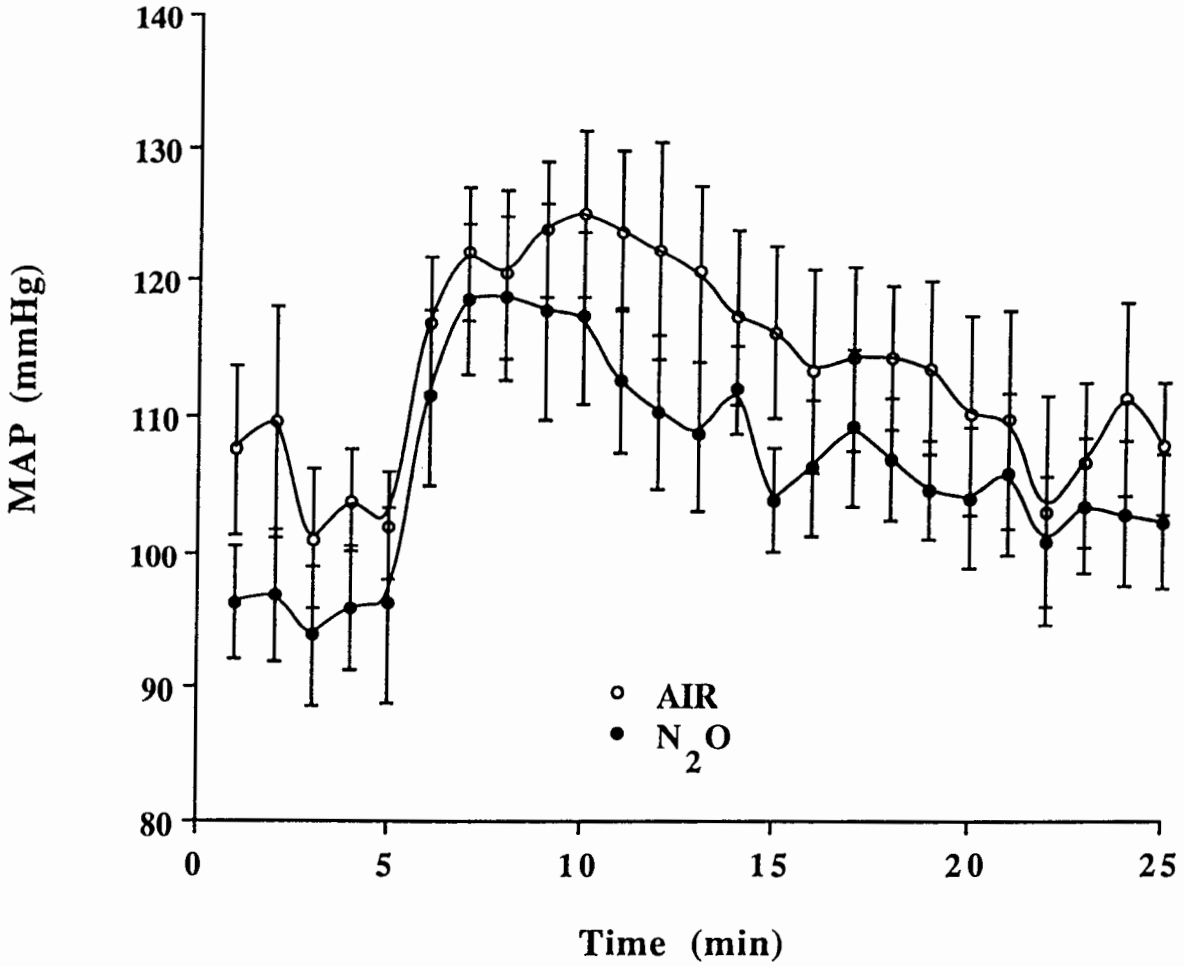

FIGURE 12: Mean arterial pressure during exercise at 50% Wmax while inhaling air or 30% nitrous oxide (N = 7; mean ± SE).

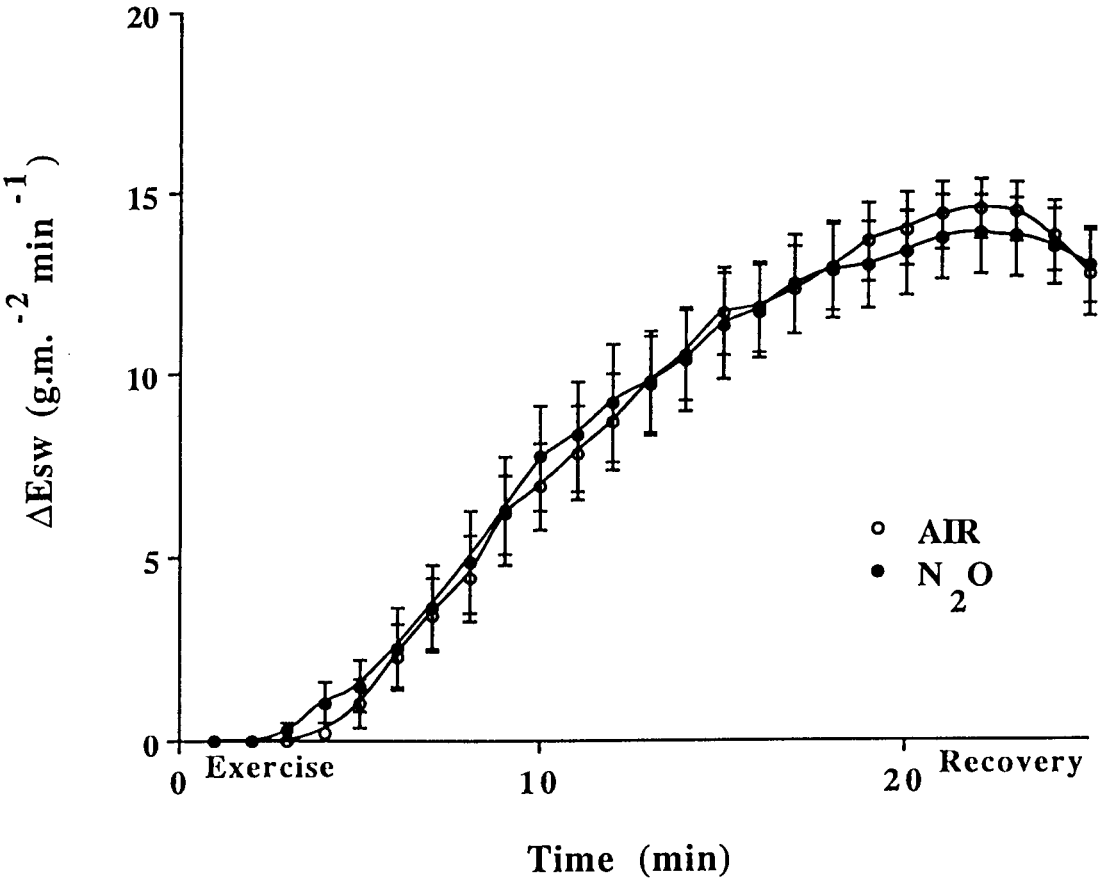

FIGURE 13: Sweating response during exercise at 50% Wmax while inhaling air or 30% nitrous oxide (N = 9; mean ± SE).

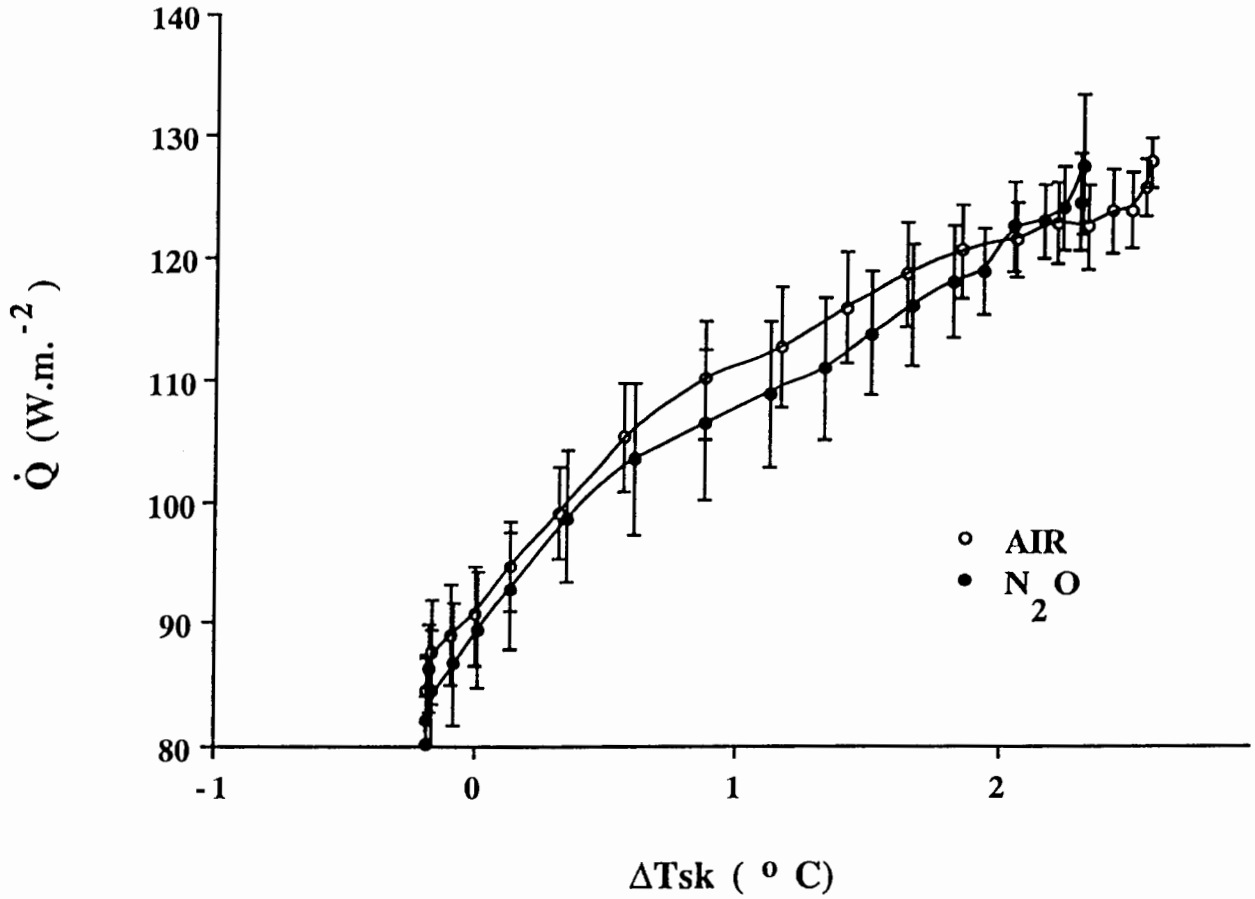

FIGURE 14: Heat flux with change in skin temperature during exercise 50% Wmax while inhaling air or 30% nitrous oxide (N = 6; mean ± SE).

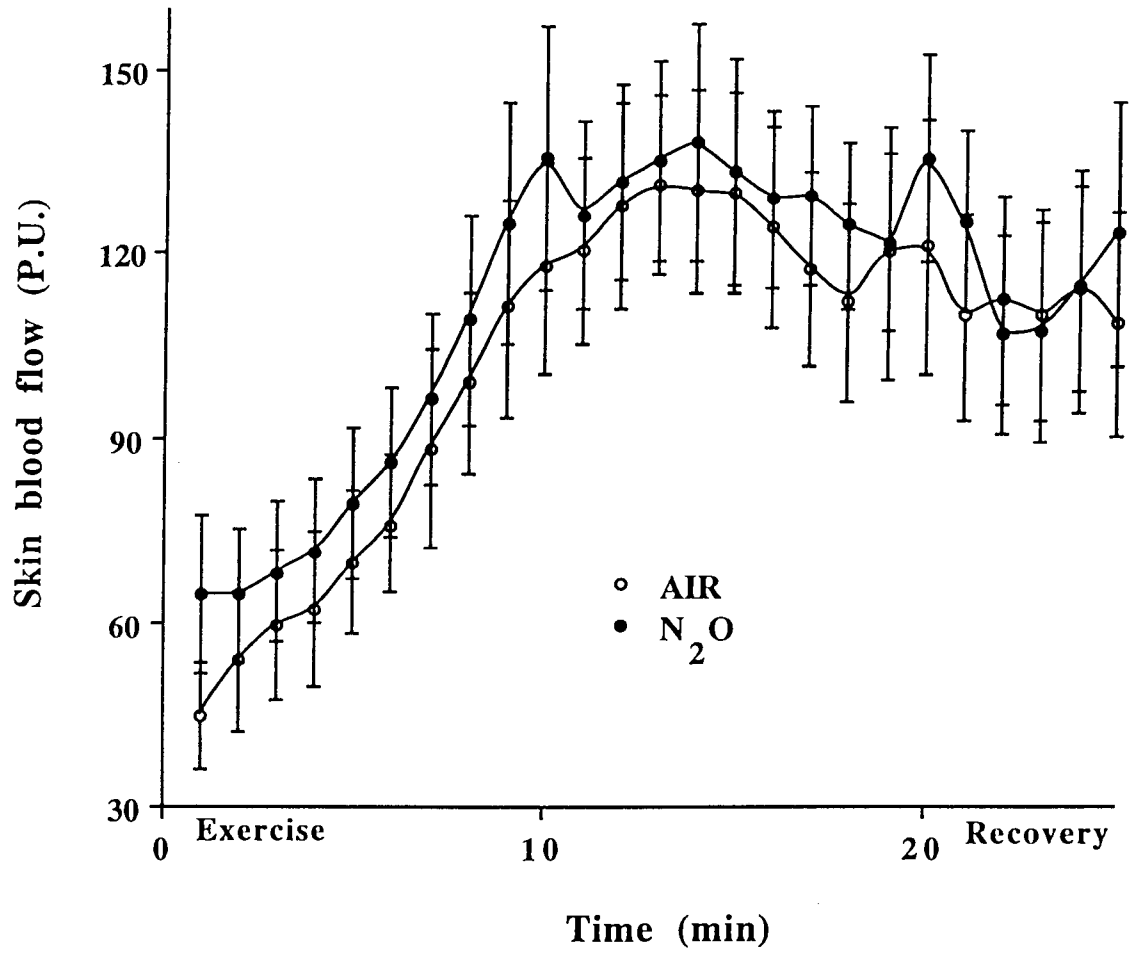

FIGURE 15: Cutaneous blood flow (perfusion units) during exercise at 50% Wmax while inhaling air or 30% nitrous oxide (mean ± SE).