

To the Graduate Council:

I am submitting herewith a thesis written by Alan G. Brice entitled "The Metabolic Responses to Helium-Oxygen Breathing during Exercise." I recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Zoology.

Hugh G. Welch
Hugh G. Welch, Major Professor

We have read this thesis and
recommend its acceptance:

Edward T. Howley
James N. Giles

Accepted for the Council.

L. Evans Pugh
Vice Chancellor
Graduate Studies and Research

THE METABOLIC RESPONSES TO HELIUM-OXYGEN
BREATHING DURING EXERCISE

A Thesis

Presented for the

Master of Science

Degree

The University of Tennessee, Knoxville

Alan G. Brice

December 1980

3046753

ABSTRACT

In order to compare the metabolic responses of helium-oxygen breathing during exercise with those of air breathing, four trained male cyclists performed exercise at six different intensities on the bicycle ergometer. Each subject was tested twice at each work load, once while breathing air and once while breathing He-O₂. Only one test was performed per day and tests were separated by a period of approximately one week to minimize any training or fatigue effects. The variables measured were: oxygen uptake, blood lactate concentration, minute ventilation, and heart rate.

Only the variable minute ventilation showed a statistically significant difference between the breathing mixtures. There was also a significant interaction between work rate and gas for the variable minute ventilation. Tests for simple main effects showed no differences in ventilation between the gases at the low work rates but highly significant differences at the more intense work rates.

There were no statistically significant differences between the breathing mixtures for oxygen uptake or lactate. However, there were definite trends toward higher oxygen uptakes at the high work rates when inspiring He-O₂. Lactate also demonstrated a tendency to be higher on He-O₂. The ambiguous nature of these results suggests the need for further work on these questions.

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CHAPTER I

INTRODUCTION

Although helium (He) has been employed in respirable atmospheres as the diluent gas in place of nitrogen (N_2) for years, the biophysical effects of this rare gas on man and other biological systems remain relatively unsettled and poorly understood (2, 3, 26, 34, 49, 51). Because the use of artificial breathing mixtures utilizing He as the diluent gas is increasing, it is important to pursue further the question of the substitution of He for N_2 .

Helium is used as the diluent gas in mixtures breathed by humans working under high atmospheric pressures such as those which would be encountered during undersea oil exploration, tunneling operations, caisson work, and recreational diving (2). The lowered solubility of He relative to N_2 in blood, lipids, and other tissues reduces the time required for decompression of those working under high pressure conditions (46). The N_2 in air at 5-10 atmospheres pressure produces narcotic effects in man. These effects are a semi-loss of consciousness, feelings of euphoria, and a reduction of intellectual and perceptive facilities (52). This phenomenon is known as "nitrogen narcosis," "inert gas narcosis," or more commonly as the "rapture of the deep." Because of its supposed inertness and low solubility in lipid, the narcotic effect of He should be negligible at pressures which cause N_2 narcosis. In fact, experiments have shown that a pressure of 163 atmospheres of He is required to produce central nervous system effects in mammals (52).

Helium, therefore, is the gas of choice in deep-sea, high pressure operations (52).

Helium has also found widespread use in medicine. The substitution of He for N_2 yields a respirable gas mixture that is 33% as dense as air (46). Because of this low density, these mixtures are used in the treatment of patients with severe asthma and other obstructive respiratory diseases since the force required to move the He- O_2 mixture is lower, relative to air. Helium is also used in anesthesia as its inertness is useful in the prevention of fires and explosions which could arise from the flammable nature of the mixtures used in anesthetic practice (46).

Considerable study has been given to the effects of replacing N_2 with He in the respired gas, both at high pressure and subatmospheric conditions. The influence of the inert gases on metabolism has been under investigation for years, but no clear answers concerning the effect of He on living systems are yet available. From the investigations in which He and other noble gases have been studied, we are presented with a wide variety of possible physiological alterations these gases may induce. There have been reports that He stimulates metabolism (7, 8, 13, 28, 30, 42, 43, 49, 51, 53, 54), depresses metabolism (6, 7, 26, 30), or has no effect whatsoever (3, 12, 48). In fact, there are some investigators who strongly feel that the biological effects of He are so insufficiently understood that it should not be used as the diluent in artificial atmospheres—because the use of He in these respirable gases may present physiological hazards (22, 26).

Research on humans in which He has been substituted for N_2 in the inspired gas mixture has thus far been mainly concerned with problems

associated with decompression sickness and inert gas narcosis (11). These studies were conducted under high atmospheric pressures. The physiological effects of He on humans at one atmosphere remain poorly defined as they have received little study.

Because the use of inert gases is increasing, it is necessary that we acquire a more thorough understanding of the physiological alterations in normal body function that may be induced through the inspiration of He-O₂ mixtures. It will be the purpose of this investigation to establish whether He at one atmosphere can alter metabolism in man while exercising at various intensities. The use of various intensities of exercise will permit the evaluation of the effects of He on various levels of metabolism in case the presence of He selectively influences physiological functions only at certain levels of metabolism.

CHAPTER II

REVIEW OF RELATED LITERATURE

The general effects of He on metabolism are unclear. A survey of the literature reveals a wide variety of physiological alterations attributed to the gas. There are many discrepancies as to what exactly are the effects of He on the intact organism and at the cellular and molecular level. If there are indeed real physiological changes occurring when N_2 in the respired gas is replaced by He, the mechanisms of these changes are also not clearly defined. This review of the literature will be arranged into the following areas: Physical Properties; Effects on Intact Animals; Effects on in vitro Tissue Preparations; Effects on Sympathetic Nervous System and Cardiovascular Function; and Effects on Humans.

Physical Properties

Helium is classified as one of the Group 0 elements on the Periodic Table and is often classified as being a member of the "rare gases," "inert gases," or "noble gases." Helium exists in the monoatomic form (He) rather than the diatomic form (He_2) and has an atomic weight of 2.0000. The two electrons He possesses give this element a complete 1S electron shell, thereby making this species very chemically unreactive (33). The valence of He is normally zero (59).

Of physiologic importance besides He's supposed chemical inertness are some other physical properties, namely its viscosity, density, and

high thermal conductivity. The viscosity of 21% oxygen (O_2) in He at normal ambient temperature is 1.1 times that of air (15, 32, 42, 59). The density of a 21% O_2 -79% He gas mixture is .34 times that of air (15, 32, 56). Lastly, the gas has an enormous heat conductivity which is approximately 6.5 times greater than that of N_2 (3, 27, 34, 59).

Effects on Intact Animals

Metabolic disturbances occurring in intact animals exposed to He- O_2 environments have been reported in the literature. There presently exist two general theories to explain the physiological changes brought about by He. The first pertains to mammals and is known as the "indirect theory." This states that when a temperature gradient exists between the skin and the ambient gas, the six-fold greater heat conductivity of He promotes heat loss from the body surface (43). This heat loss results in a slight fall in body temperature which in turn causes the animal's homeothermic mechanisms to increase metabolism to permit maintenance of a constant body temperature (19, 20, 27, 43, 49, 51). The "direct theory" states that He has a direct pharmacological effect, acting on enzyme systems and oxidative processes at the cellular and molecular level (7, 28, 30, 42, 43, 49, 51, 53, 54).

Cook (6), in 1950, provided the first information on the metabolic effects of He at one atmosphere on a variety of animals. He found that when He is substituted for N_2 at one atmosphere no changes occurred in the O_2 consumption of yeast, fruit flies, termites and lizards. However, He increased O_2 consumption in the larvae and pupae of meal worms.

Development time of fruit flies and meal worms decreased following exposure to He. Oxygen consumption of mice was not measured but their CO_2 output was increased, indicating an accelerated metabolic rate. Since this initial study was exploratory in nature, Cook offered no explanation to account for these results; however, he stated in conclusion that He is "not inert in the physiological sense but can alter the rate of several fundamental vital processes."

In conflict with Cook's (6) report of an accelerated metamorphosis in fruit flies and meal worms is the study of Frankel and Schneiderman (12). These investigators found that when He or argon (Ar) was substituted for N_2 at one atmosphere no changes in the rate of development of these insects at any stage occurred. They concluded that inert gases at one atmosphere pressure do not accelerate the development of these organisms. However, they reported that at 5 and 10 atmospheres of pressure N_2 and Ar did retard development whereas He did not.

In studies involving the O_2 consumption of mice, Cook et al. (8) and Galvin et al. (13) found a significant increase in O_2 uptake when He was substituted for N_2 as the diluent gas. Galvin et al. suggested the N_2 at a normal pressure of .8 atmosphere may "exert a braking influence on metabolic processes." Young and Cook (63) attempted to correlate the acceleration of metabolism by He with the corresponding level of metabolism in air by varying the standard metabolic rate. They accomplished this by either removing the thyroid or injection of excess thyroxin into the experimental animal. They found the increase in O_2 uptake resulting from He exposure to be greatest in the thyroidectomized

animals with their concomitant low resting metabolic rate and least in the animals receiving supplemental thyroxin. Young and Cook state that the "effect of He is inversely proportional to the level of standard metabolism."

Leon and Cook (27) first suggested the "indirect hypothesis" to explain the observed increases in metabolism when exposed to He. They found that the high thermal conductivity of He-O₂ relative to air is "of a magnitude more than sufficient" to account for the increased O₂ uptake reported in the earlier studies. When ambient temperature was near skin temperature the effect of He was negligible. The authors concluded that metabolic increases upon exposure to He occur to compensate for the increased heat loss and that "the magnitude of this response is proportional to the thermal gradient between the animal and environment." The results of numerous other studies are also in accord with this viewpoint. When the thermal properties of He-O₂ environments were negated by raising ambient temperature, He was found to have no effect whatsoever on metabolism (19, 20, 43, 49, 51).

It should be noted that as much sense as the increased heat conductivity concept makes for explaining the He effect in mammals, it is hard to explain the changes in metabolism occurring in tissue slices (discussed in next section), insects (6), and seeds (26) where mechanisms for maintaining homeothermia do not exist. Rhoades et al. (43) feel that He may still have a direct "pharmacologic action on poikilotherms and isolated tissues which is lost or masked in intact homeotherms." It is apparent that this issue is far from being resolved.

Before ending this discussion on the effect of He on intact animals, mention should be made of the several studies indicating that He as a constituent of ambient atmospheres may be harmful. Weiss et al. (60) found embryo development and chick growth to be retarded in a He-O₂ atmosphere, but the authors attributed an increased conduction of heat as being responsible for the observed effects, not a pharmacologic action of the gas. Hawkins (22) found that mice exposed for 90 days to 80% He- 20% O₂ at one atmosphere developed myopathic lesions in skeletal muscle—indicating that muscle metabolism was affected. This finding has not been confirmed in any other known published reports.

In summary, although both short and long term exposure to He seems to be harmless, a wealth of information exists to implicate He in an alteration of metabolism. Whether the alteration is indirectly due to the physical properties of this gas or to a direct effect at the molecular level is unknown at this time.

Effects on in vitro Tissue Preparations

As was discussed in the previous section, the accelerated metabolism reported in small mammals exposed to a He-O₂ environment is likely due to the greater heat loss which occurs in the presence of He (19, 20, 43, 49, 51). However, this "thermal" explanation may not always be correct. Reports of changes in O₂ consumption occurring with in vitro tissue preparations as a function of the inert gas present represent a phenomenon for which a thermo-homeostatic mechanism cannot be invoked.

Cook et al. (8) measured the O₂ consumption and CO₂ production of diaphragm (striated muscle) and liver slices by conventional technique

in Warburg vessels. Exposure to 80% He-20% O₂ at one atmosphere resulted in a statistically significant increase in O₂ uptake and CO₂ production. Although the authors proposed no mechanism for this increase, they concluded that He at one atmosphere accelerates respiratory metabolism in mammalian tissues. In a continuation of the preceding study, Cook and South (7) examined the effects of He-O₂ at one atmosphere on brain and sarcoma slices. Standard Warburg techniques were once again employed. Helium increased the rate of O₂ consumption in both tissue types with the greatest increase occurring in the sarcoma tissue. Anaerobic glycolysis was found to be depressed in the brain slices but not the sarcoma tissue. In an attempt to explain this finding, the authors suggested that He may somehow alter the rate of phosphorylation of glucose during anaerobic conditions.

In view of their previous findings that He increased the O₂ consumption in liver, brain, diaphragm, and sarcoma slices, South and Cook (53) investigated the effect of He on specific energy yielding pathways in respiration and glycolysis using mouse liver slices. Under aerobic conditions (80% He-20% O₂), the investigators discovered that the rate of O₂ consumption and CO₂ production increased equally. This constant respiratory quotient between the He-O₂ mixture and air implies that the same substrate is being metabolized in He-O₂ as in air. They also noted that He tended to alleviate the depression of the cytochrome oxidase system by cyanide. The tricarboxylic acid cycle in tissue slices was unaffected by He but was altered unexplainably in tissue homogenates. In conditions of anaerobiosis (95% He-5% CO₂), glycolysis was inhibited

at some point above the aldolase reaction and accelerated between the aldolase and enolase reactions. In a similar study, Rinfret and Doebbler (46) reported that He inhibited phosphorylation reactions, interfered with the cyanide-cytochrome oxidase system, and increased O_2 consumption and CO_2 production of in vitro preparations.

Other investigators have not agreed with the above findings (30, 48). Rodgers et al. (48) found that the presence of N_2 or He under normoxic conditions at one atmosphere failed to alter the rate of O_2 consumption in rat diaphragm and slices of liver, brain cortex and kidney cortex. This observation is substantiated by Maio and Neville's (30) finding that no significant differences in O_2 consumption rates of rat liver slices occurred when exposed to He- O_2 instead of air. Rodgers et al. (48) and Schreiner (51) offered the following explanation to account for the discrepancies on this issue. Rodgers et al. found that changes in the partial pressure of O_2 (PO_2) in the range of .204 to .262 atmospheres can produce significant changes in the O_2 consumption of the tissue preparations. In their own experiments great care was taken in the preparation of the gas mixtures to ensure a constant PO_2 in all experiments. Schreiner and Rodgers et al. are of the opinion that the methods used to prepare the gas mixtures in the earlier studies of South and Cook (7, 8, 53) may not have been precise enough to give a constant PO_2 in each and every experiment since a change in PO_2 of only .06 atmosphere could significantly affect O_2 consumption.

Other evidence exists to support the claim that inert gases are physiologically active. Maio and Neville (30) found reductions in O_2

uptake by yeast and rat liver slices in the presence of He, N₂, and Ar relative to the O₂ consumption of these preparations in 100% O₂. At first glance one might speculate that these changes could be completely attributable to the differences in PO₂. However, the authors found that the O₂ uptake of tissue homogenates (rather than whole tissue slices) was not decreased by these inert gases. Maio and Neville hypothesize that in the intact tissue slices the inert gases may act on a membrane mechanism for O₂ transport into the cell. Schatte et al. (49) are also of the opinion that the diluent gas may somehow influence O₂ availability to the cell.

It should be apparent that the question of whether He exerts an effect on metabolism at the cellular and molecular level is still unresolved.

Effects on Sympathetic Nervous System and Cardiovascular Function

The possible effects of He on sympathetic nervous system and cardiovascular function gained considerable attention from the observation of Pifarré et al. (38) that breathing He lowered the incidence of cardiac arrhythmias following ligation of the left coronary artery in anesthetized dogs. Pifarré et al. (39) and Raymond et al. (42) repeated this finding. In addition, Raymond et al. found that He reduced baseline heart rate (HR) and the concentration of endogenous plasma catecholamines. Gledhill et al. (16) also reported a reduction in plasma epinephrine (E) and norepinephrine (NE) during He inhalation by humans and a restoration of normal catecholamine levels 10 minutes from the cessation of He

breathing. They concluded that He inhalation alters catecholamine activity in man at rest. These findings make it appear likely that the sympathetic nervous system could be a site for a direct pharmacologic action of He (34).

To test this hypothesis Nicholas et al. (34) investigated the effect of 80% He-20% O₂ on sympathetic function in rats. They found that both unanesthetized and pithed rats responded to injected NE (the post-ganglionic transmitter), tyramine (used as an index of the readily releasable stores of NE), and dimethylphenylpiperazinium (a ganglionic stimulating agent) similarly in He-O₂ and air. Helium was not found to alter the turnover of cardiac NE. In conflict with the findings of Raymond et al. (42) and Gledhill et al. (16), Nicholas and associates found no changes in plasma catecholamines attributable to He breathing, but the authors pointed out that a change of less than 1 ng/ml would have escaped detection in their preparation. In agreement with Nicholas et al., Hoar et al. (23) found no He-induced changes in urinary catecholamine levels. In this study, scuba divers did ergometric work at a depth of 3 meters while breathing air or a helium trimixture (36% He-21% O₂-43% N₂). As expected, they found that urinary E and NE rose significantly during work, but that the increase was the same for both gas mixtures. The authors concluded that He had no effect on E and NE excretion.

Leon and Harris (28) reported that the breathing of He increased the minimal directly applied electrical stimulus needed to produce ventricular fibrillation in dogs. They attributed this phenomenon of

an elevated ventricular threshold to a direct myocardial action of the gas. However, He did not affect arterial PCO_2 , PO_2 , or pH, atrioventricular conduction times, HR, arterial pressure, cardiac output, or stroke power. Boerboom and Boelkins (2), using awake rabbits breathing 20% O_2 -80% He at one atmosphere, reported results similar to those of Leon and Harris. Blood pressure, cardiac output, systemic vascular resistance, organ blood flow, and resistance to flow were found not to be altered by He. The only change noted was a 13% decrease in HR after two hours of He breathing. The authors concluded that the cardiovascular system is not adversely affected by He.

A He-induced influence on the sympathetic nervous system could also be reflected by a change in HR (34). Fox and co-workers (11), when investigating the thermal responses of man during rest and exercise in a He- O_2 environment, found no differences in HR attributable to He inhalation. Bowers and Fox (3) reported that HR is "independent of the gas mixture breathed in resting subjects." Hoar et al. (23), in the study employing exercising scuba divers, also found no change in HR.

There are other reports in the literature that He exerts no effect on HR (28, 41, 55, 56). There is disagreement in the literature about this issue as there are papers reporting a decrease in HR when He is inspired (2, 29, 42). Lin and Kato (29), using unanesthetized rats, found a significantly lower HR in He- O_2 than in air at any given O_2 uptake. The authors attributed this lowered HR to an elevation of vagal tone and a depression of sympathetic influence on the heart when breathing He- O_2 .

Effects on Humans

There is an abundance of information contained in the literature concerning the effects of inert gases at high pressure on man, but this topic will not be dealt with in this literature review. Instead, studies concerned with the thermal response of man in a He-O₂ environment at one atmosphere pressure and long term exposure to He will be briefly discussed. This will be followed by a detailed review of the limited literature pertaining to the response to exercise while breathing He.

Fox et al. (11) investigated the thermal responses of man in a He-O₂ environment during rest and exercise. They discovered that when a temperature gradient existed between the skin and ambient atmosphere, mean skin temperature was lower by 1.0 F at rest and 2.3 F at the end of exercise, indicating an increased heat loss due to the high thermal conductivity of He. However, no significant increases in metabolic rate were noted. Rhoades et al. (43) suggested that mechanisms such as vasoconstriction and control of evaporation can compensate for the increased heat loss in He-O₂. Bowers and Fox (3) also observed a decrease in the mean skin temperature of resting subjects exposed to He-O₂. In this study, O₂ consumption, HR, pulmonary ventilation, and the respiratory exchange ratio were found to be independent of the gas breathed, implying no alterations in metabolism.

In order to assess the physiologic suitability of various atmospheres for manned spacecraft, Hargreaves et al. (21), Zeff et al. (64), and Robertston and McRae (47) investigated the effects of a 56-day exposure to a He-O₂ atmosphere at 258 mm Hg total pressure. In summary, these

investigators found no changes to indicate that this atmosphere would impair pulmonary function or cardiovascular response. Hargreaves et al. stated: "Helium could be an acceptable substitute for nitrogen in the inspired gas mixture and that no adverse effects should be noted when this substitution is made." It should be pointed out that the partial pressure of He in these experiments was only 74 mm Hg. It may be possible that this partial pressure is below a critical threshold required to induce any physiological alterations.

Tait (56) investigated exercise performance in 4 subjects breathing air or He-O₂ and found a slightly higher O₂ consumption per kg of body weight when subjects inspired He-O₂. Although this increase was non-significant, there remains the distinct possibility that had more subjects been utilized the observed increase in O₂ uptake may have become significant. Fagraeus (10) demonstrated an increase in the O₂ consumption ($\dot{V}O_2$) of exercising subjects under hyperbaric He-O₂ conditions. Whether this increase in metabolism is completely ascribable to He is doubtful as the increase may be due in part to the three-fold increase in the PO₂ of the inspired gas mixture in this hyperbaric environment. Raymond et al. (41) reported no changes in $\dot{V}O_2$ or in end-tidal CO₂ levels of subjects performing mild exercise when breathing air or a 40% He-21% O₂-39% N₂ mixture.

To further cloud this issue of changes in O₂ consumption occurring when He is inspired are reports that breathing He-O₂ decreases the $\dot{V}O_2$ for a given work rate relative to air (23, 32, 55). In a previously cited study, Hoar et al. (23) found that scuba divers performing ergometric

work had a lower $\dot{V}O_2$ breathing He trimixture than when breathing air. The authors speculated that this decreased $\dot{V}O_2$ is related to a decreased work of breathing with He trimixture as its density is less than that of air. Murphy et al. (32) and Spitler et al. (55) reported similar observations. These studies will be discussed subsequently.

The hypothesis of a reduced O_2 cost of breathing when inspiring a less dense gas such as He- O_2 is not unreasonable when one considers the O_2 cost of breathing during exercise. Under resting conditions the O_2 cost of breathing is relatively small. The muscular work done by the respiratory muscles during quiet breathing is probably not much more than 1% of the resting metabolism (9). Bartlett and Specht (1) calculated the cost of resting ventilation to be .5 ml O_2 per liter of ventilation. They found that as ventilation increased the O_2 consumption required for the increased ventilation rose exponentially. McKerrow and Otis (31) reported that the cost of maximal voluntary ventilation may become extremely high, as much as 2 liters of O_2 per minute. They also feel that as the upper limit of ventilation is approached, the cost of breathing rises very rapidly with little increase in ventilation.

Bradley and Leith (4) reported that there are wide variations in the O_2 cost of breathing among subjects. Using a linear regression equation, the authors calculated that nearly 900 ml O_2 per minute would be required by the respiratory muscles to ventilate 180 liters of air per minute.

Riley (45) believes that the O_2 cost of breathing may be so high during maximal exercise that "a point of diminishing returns is reached with regard to the amount of O_2 available to exercising muscles." He suggested that any rise in O_2 consumption due to increases in ventilation may be entirely consumed by the respiratory muscles themselves. Riley postulated that once cardiac output has reached its limit, the " O_2 cost of breathing would dominate the total cost of tissue maintenance and O_2 transport" and "could reasonably be considered the limiting factor." Ouellet et al. (37) also suggested that the O_2 available to exercising muscle does not increase once cardiac output has reached its maximum; all additional O_2 resulting from increased ventilation is utilized by the respiratory muscles. Otis (35) calculated that at ventilations over 140 liters per minute all additional O_2 provided is consumed by the respiratory muscles.

An 80% He-20% O_2 breathing mixture is one third as dense as air so it would seem that the respiratory muscles should work less when moving a given volume of He- O_2 relative to air. This should be reflected by a decrease in the O_2 cost of breathing on the He- O_2 mixture. Also, a more dense gas will cause flow through the tracheo-bronchial tree to be turbulent rather than laminar so the work of breathing will be higher for the more dense gas (15). The work of Murphy et al. (32) is in accord with this viewpoint. These investigators argued that breathing the less dense He- O_2 mixture would decrease the amount of turbulent flow in the airways which in turn will reduce the muscular work required

for a given ventilation. They state that the O_2 cost of breathing $He-O_2$ will be less than that of breathing air at high ventilations and that this should lead to a measurable decrease in the O_2 cost of intense exercise. The results of Murphy et al. agree with this prediction. At low work rates (and their corresponding low ventilations) there were no differences in $\dot{V}O_2$ between the breathing mixtures. At the higher work rates the $\dot{V}O_2$ for $He-O_2$ fell below that of air, indicating that the O_2 cost of breathing was less for the $He-O_2$ mixture. In a recent study using both men and women as subjects, Spitler et al. (55) also found a reduction in $\dot{V}O_2$ when the subjects breathed $He-O_2$. These reductions in $\dot{V}O_2$ were significant only at the maximum work rates. The authors attribute this decrease to the low density of $He-O_2$, its decreased resistance to flow, and the concomitant decrease in the energy cost of breathing.

As with almost everything discussed thus far, there is conflict in the literature as to what effect the breathing mixture will have on minute ventilation. Murphy et al. (32) found the ventilation to be identical for the two breathing mixtures, even at the intense work rates. Other investigators reported similar findings (3, 41). On the other hand, Spitler et al. (55) and Tait (56) found minute ventilation on $He-O_2$ to be similar to those of air at low work rates but the ventilation values were progressively higher at the more intense work rates. Wilson and Welch (62) reported that during maximal exercise, minute ventilation was considerably higher when the inspired gas was $He-O_2$ instead of air. There are other reports in the literature of higher minute ventilations when the subject inspires $He-O_2$ rather than air (23, 57, 58).

In a study comparing respiratory gas exchange when breathing He-O₂ or air during exercise and rest, Guenard et al. (17) found no difference in the arterio-alveolar differences for O₂ and CO₂ under normoxic conditions. Expired PCO₂ and PO₂ curves expressed as a function of expired volume were also similar between the two gas mixtures.

The subjects participating in the study of Fagraeus (10) reported that they found it easier to perform the maximal work test in the hyperbaric He-O₂ environment than in an air environment at one atmosphere. When exercising at a rate that would cause exhaustion in 3-5 minutes, the endurance times were significantly longer when in the hyperbaric He-O₂ environment. It is possible that the increased PO₂ of this hyperbaric environment, rather than an effect of He, played the major role in this increased performance time. The subjects of Zeff et al. (64) reported less difficulty in pedalling the ergometer in a hypobaric He-O₂ environment, but the hypobaric condition may have played a role in this subjective feeling. Wilson and Welch (62) found significantly longer running times on the treadmill when subjects breathed normoxic He-O₂ instead of air. When their subjects ran at a speed that required 110% of their $\dot{V}O_{2\max}$ performance times increased by approximately 20 seconds. Tait (56) also reported that mean maximal treadmill running time was nonsignificantly longer when the subjects breathed normoxic He-O₂ instead of air. Again, before putting too much weight on this finding one must remember that Tait used only 4 subjects. Spitler et al. (55) reported no change in endurance times between air and He-O₂.

There are several hypotheses to explain these reported performance increases. If the O₂ cost of breathing He-O₂ is less than that of air,

the O_2 that is "spared" can be utilized by the exercising skeletal muscles. This could possibly raise the anaerobic threshold. Wilmore (61) defines anaerobic threshold as "that point where the metabolic demands of exercise cannot be met totally by available aerobic sources and at which an increase in anaerobic metabolism occurs." If this threshold is indeed raised, the resulting decrease in lactic acid production could conceivably prolong the time before metabolic acidosis would cause exercise to stop. The large difference in ventilation reported between the two breathing mixtures at the higher work rates could cause significant changes in the acid-base status of the body. The large ventilations occurring when breathing He- O_2 may aid in buffering the metabolically produced acids via respiratory compensation. The last possibility is that N_2 may exert a narcotic effect and this depressant effect is removed when N_2 is replaced by He as the diluent gas.

It would seem that if alterations in metabolism do occur in response to He, these alterations might be reflected by changes in lactate production. Strangely enough, in all the studies cited in which subjects breathed He- O_2 at one atmosphere, blood lactate was not measured.

In conclusion, it is apparent from this review of the literature that there are few if any clear answers to the questions of He's effect on metabolism, whether they be direct or indirect.

CHAPTER III

METHODS

Three sets of experiments were performed. In Set 1, ventilation ($\dot{V}$), heart rate (HR), $\dot{V}O_2$, $\dot{V}CO_2$ and lactate were measured at 4 levels of exercise during air and He- O_2 breathing. In the second set (Set 2), the same measurements were made as in Set 1 but exercise was performed at only 2 levels of intensity. For Set 3 only 1 work rate was utilized. In the following description of methods, any differences in protocol among the sets will be noted.

Subjects

Four healthy, male volunteer subjects participated in this study. The physical characteristics of each are given in Table 1. The subjects were individuals who were familiar with the laboratory surroundings, experienced in the maintenance of constant work rates on the bicycle ergometer and who were accustomed to the levels of activity required in the study. This requirement was critical because it was necessary that the subjects be able to maintain the required work output precisely for the periods of time required in the study. Informed consent was obtained prior to participation in this study.

Maximal Oxygen Uptake Test

Each subject participated in a preliminary maximal oxygen uptake ($\dot{V}O_2$ max) test administered on a Monark bicycle ergometer prior to the beginning of the experiments. The work rates used in the experiments

Table 1. Physical characteristics of subjects.

Subject	Age (Years)	Weight (kg)	$\dot{V}O_2\text{max}$ (l/min)	$\dot{V}O_2\text{ max}$ ($\text{ml}/\text{kg}\cdot\text{min}$)
1	26	80.0	4.45	56
2	42	68.0	3.12	46
3	26	65.0	3.67	56
4	29	61.0	3.75	61

were based upon the $\dot{V}O_2$ max value obtained for each subject. The $\dot{V}O_2$ max test was a progressive test in which a constant pedal frequency of 72 revolutions per minute was utilized. The resistance was increased in increments of .5 kg at 4 minute intervals until exhaustion. Expired gas samples were collected during the last 2 minutes of each resistance setting. From the expired fraction of O_2 , the $\dot{V}O_2$ was calculated. The criterion of a plateau in O_2 uptake in response to an increase in work rate was used in determining the $\dot{V}O_2$ max. The highest value achieved was taken to be the subject's $\dot{V}O_2$ max. The values for $\dot{V}O_2$ max are given in Table 1.

Gas Mixtures

Two gas mixtures were utilized in this study, air and He- O_2 . The actual mean percentages of the gas mixtures utilized in Set 1 for air and He- O_2 respectively, were 79.03% N_2 -20.90% O_2 -.07% CO_2 and 79.04% He-20.96% O_2 -0% CO_2 . In Sets 2 and 3 the values were 79.08% N_2 -20.92% O_2 -0% CO_2 for air and 78.97% He-21.03% O_2 -0% CO_2 for He- O_2 . As can be seen, the only difference in composition between the two mixtures is that He has been substituted for N_2 as the diluent gas.

In Set 1, the gas mixtures were stored in a 960-liter bag made from aluminum and polyethylene (Scotch-Pak, 3-M Corporation). The mixtures were prepared by adding either room air or the proper amounts of He and O_2 to a 120-liter Tissot spirometer. The bag and spirometer were connected together and the gas mixture washed back and forth between the bag and spirometer to ensure adequate mixing and humidification. The

mixtures in the bag were analyzed before and after each experiment. The concentration of the mixtures was found not to change during the experiment.

For the experiments in Sets 2 and 3, the air mixture was obtained from commercially filled compressed gas cylinders. The He-O₂ mixture was made in the laboratory from pressurized cylinders of commercially supplied He and O₂. The He-O₂ tanks were stored on their sides and rolled before use in experiments to mix the gases and minimize the chance of layering that might have occurred due to density differences between O₂ and He. It was determined that this precautionary measure was unnecessary as the gases remained well mixed regardless of how the cylinders were stored. During Sets 1 and 2, the order of assignment of gas mixtures to the subjects was random and the subject was not informed of the nature of the gas being breathed.

Performance of Exercise

Subjects exercised on a Monark bicycle ergometer that was calibrated before each experiment to ensure constant work rates from test to test. Pedal frequency was 72 revolutions per minute and was timed by a metronome. Checks were made periodically of pedal frequency during the experiment by one of the technicians using a stopwatch. As all the subjects were experienced cyclists, no problems were encountered in maintaining the desired frequency.

For Set 1, each subject exercised at 4 intensities ranging from very light to very intense. The work rate at which each subject exercised

was based upon his $\dot{V}O_2$ max. The 4 intensities required approximately 40, 60, 75 and 100% of the subject's $\dot{V}O_2$ max. The work rate requiring 100% of $\dot{V}O_2$ max could be maintained for only 3 to 4 minutes.

During Set 2, each subject exercised at 2 intensities, one requiring approximately 70% of the subject's $\dot{V}O_2$ max and the second requiring approximately 90% of $\dot{V}O_2$ max, which could be maintained for 4 to 5 minutes.

One subject participated in the experiments of Set 3. The work rate utilized was 149 watts and required approximately 60% of this subject's $\dot{V}O_2$ max.

Blood Sampling Procedure

All blood samples obtained in this study were drawn via fingertip puncture. Upon completion of each experiment in Set 1, the subject's hand was submersed in warm water for 2 minutes. In Sets 2 and 3, the subject's hand was wrapped in a heating pad during the experiments to keep the hand and fingers warm.

Each blood sample drawn was treated in the following manner. The sample was placed in a spot plate, the well of which was coated with 50 μ l of a 4% NaF solution. This dried NaF solution served to prevent clotting and inhibit glycolysis. The blood and NaF were mixed and 2 separate 50 μ l samples were taken and each placed into 2 ml of 7% $HClO_4$. These samples were well mixed by shaking and were then centrifuged. The supernatant fluid was frozen for a later lactate analysis.

Experimental Protocol

Each subject was tested twice at each of the 6 work rates contained in Sets 1 and 2, once on the air mixture and once on the He- O_2 mixture.

On any given test day a subject was tested on only one gas mixture. In order to minimize any possible training or fatigue effects, tests were separated by a period of about 1 week. The order of assignment of the test gas was random and the subject was not aware of the composition of the test gas.

The tests were conducted under the following environmental conditions: ($T^{\circ}\text{C}$: 21-26; P_B : 730-746 mm Hg; RH: 25-62%). An electric fan was provided for use by the subject and was controlled by the subject from a switch mounted on the handle bars. Subjects were asked to report for testing in the postabsorptive state.

Regardless of which mixture was used, the following protocol was used every testing session. The subject arrived at the lab and changed into gym clothes. Disposable ECG electrodes (Medi-Trace, Graphic Controls Corporation) were placed on the chest for monitoring HR. The subject then mounted the bicycle ergometer and immediately began breathing the gas mixture via a mouthpiece attached to a low resistance Koegel breathing valve. Gas was delivered to this valve in the following manner. For the experiments of Set 1, the gas in the aluminum polyethylene bag was delivered directly to the Koegel breathing valve via a connecting 40-cm ID rubber hose. In Sets 2 and 3, dry gas from the pressurized cylinder passed through a humidifying chamber to moisten and warm the gas. It next entered a 120-liter Tissot spirometer which acted as a reservoir. From there the gas passed through a Parkinson-Cowan Model CD-4 gas meter and then directly to the breathing valve. The temperature of the inspired gas was measured by means of a Yellow Springs

Tele-Thermometer (Model 46-TUC) with a thermister inserted in the inlet port of the gas meter. Before each test the entire system was flushed by the gas to be used that day.

The experiments of Set 1 were conducted in the following manner. Once breathing on the system, the subject rested quietly for 3 minutes while sitting on the ergometer. He then began a 4-minute warm-up at a predetermined work rate. After this equilibration period, the subject exercised for 10 minutes at the submaximal work rate being used for that particular experiment. An expired gas sample was collected in a 300-liter meteorological balloon from minute 6 to 8 and another sample in a second balloon from minute 8 to 10. The exercise was halted at minute 10 and a blood sample drawn 2 to 4 minutes from the cessation of exercise for a later lactate analysis. For the work rate calculated to require approximately 100% of $\dot{V}O_2$ max, the subject followed the same warm-up procedure but exercised only for 3 to 4 minutes at this intense load. In all cases the time at this work rate was constant for a given subject between the 2 breathing mixtures. Expired gas samples were collected in 2 separate balloons, one for each of the last 2 minutes of exercise at this intense work rate. Within 2 to 4 minutes of the completion of this work bout, a blood sample was drawn for lactate analysis.

During Set 2, the subject started breathing the test gas and immediately began a 2-minute warm-up at the predetermined work rate for that subject. Following the 2-minute warm-up, the subject rested quietly on the ergometer for 3 minutes. After this equilibration period, the subject exercised at a work rate calculated to elicit approximately 70% of his $\dot{V}O_2$ max. The subject exercised for 10 minutes at this moderate

work rate. Expired gas samples were collected in 2 separate balloons from minute 6 to 8 and from minute 8 to 10. A blood sample for lactate determination was drawn at minute 7. After the 10 minutes of exercise at this moderate intensity, the work rate was immediately increased to a level requiring approximately 90% of $\dot{V}O_2$ max with no rest in between. The subject exercised at this level for either 4 or 5 minutes, depending on the subject. As was the case in Set 1, the time at this work rate was constant for a given subject between the 2 breathing mixtures. Expired gas samples were collected in 2 separate balloons for each of the last 2 minutes of exercise at this work rate. Within 1 minute of the completion of this high intensity bout, another blood sample was drawn for lactate analysis.

The only subject participating in Set 3 was tested every other day on 4 different occasions, twice on air and twice on He- O_2 . The subject breathed air during the first and fourth tests and received He- O_2 for the second and third tests. Each of the 4 tests was conducted in an identical manner. A 2-minute warm-up was immediately followed by a 3-minute rest period. The subject then exercised for 20 minutes at a work rate of 149 watts. A blood sample for lactate analysis was taken at minute 10 and another was drawn at the end of the 20-minute work bout. Expired gas samples were collected from minutes 10-12; 12-14; 14-16; 16-18 and 18-20 in 5 separate meteorological balloons.

Description of the Measurements

Oxygen consumption was determined by the open circuit method. The expired gases were analyzed using an Applied Electrochemistry Model S-3A

O₂ Analyzer. The analyzer was calibrated with standardized calibration gases previously measured by the Scholander method (50) before each sample was analyzed. These standardized calibration gases contained mixtures comparable to those being analyzed. Preliminary studies indicated that an analysis run on the electronic analyzer will agree within several one-hundredths of 1% with those of the Scholander method, even for the He-O₂ gas mixture. The O₂ consumption was calculated using the equations as described by Otis (36).

$$\dot{V}O_2 = \dot{V}I(FIO_2 - FEO_2 \cdot FIN_2 / FEN_2)$$

$$\dot{V}O_2 = \dot{V}E(FIO_2 \cdot FEN_2 / FIN_2 - FEO_2)$$

Carbon dioxide production was determined in the same manner as O₂ consumption except that the expired CO₂ fraction was measured with a Beckman LB-2 CO₂ Analyzer. Calibrations were as described above. The CO₂ production was calculated using the equations as described by Otis (36).

$$\dot{V}CO_2 = \dot{V}I(FIN_2 \cdot FECO_2 / FEN_2 - FICO_2)$$

$$\dot{V}CO_2 = \dot{V}E(FECO_2 - FICO_2 \cdot FEN_2 / FIN_2)$$

Heart rate was recorded with bipolar surface electrodes connected to a BT-1200 Biotachometer. This output was fed to the DMP-4B Physiograph to provide a continuous record of HR.

Expired minute ventilation was measured in the experiments of Set 1. The expired gas in the balloon was expelled by hand from the balloon through a Parkinson-Cowan gas meter. The volume was read directly from the dial on the meter. In Sets 2 and 3, inspired minute ventilation was measured through a Parkinson-Cowan gas meter fitted with a rotary

potentiometer. This output was fed into a servoamplifier on the DMP-4B Physiograph and provided a continuous record of ventilation, tidal volume and frequency. It was determined previously that the calibration of the Parkinson-Cowan gas meter does not change when the gas being measured changes from air to He-O₂.

Lactic acid concentration was determined by a fluorometric modification of the method of Gutmann and Wahlfeld (18). The samples were analyzed in duplicate. A Turner Model 430 Spectro-fluorometer was utilized in this enzymatic assay.

Statistical Analysis

The data of Sets 1 and 2 were combined and analyzed statistically with a 2-way ANOVA for repeated measures over two factors, work rate and gas. A 2-way ANOVA for repeated measures was utilized to test for significant differences in Set 3.

CHAPTER IV

RESULTS

Oxygen Uptake

For the experiments of the first two sets, the combined mean data for all subjects in both the air and He-O₂ treatments for the variable $\dot{V}O_2$ are listed in Table 2. The $\dot{V}O_2$ increased significantly ($p < .001$) in response to increasing work rates (see Table 3). Oxygen uptake did not differ significantly ($p = .08$) between the two experimental treatments when tested at the .05 level of significance. The interaction between gas and work rate was also found to be nonsignificant ($p = .09$). Despite the fact that no statistically significant differences in $\dot{V}O_2$ occurred due to the experimental treatment, the $\dot{V}O_2$ values were higher during the He-O₂ treatment 16 out of 24 times. This is demonstrated in Figure 1.

The mean $\dot{V}O_2$ value in Set 3 during air breathing was 2.17 liters of O₂ per minute and that for He-O₂ was 2.20 liters of O₂ per minute. The differences in $\dot{V}O_2$ values between the gas treatments were not statistically significant ($p = .13$) as shown in Table 4.

Blood Lactate

The combined mean data on all subjects for the variable blood lactate are given in Table 5. As expected, in the experiments of Sets 1 and 2 lactate concentration increased significantly ($p < .001$) with increasing work load (see Table 6). Blood lactate concentration did not differ

Table 2. Combined mean values for all subjects at each work rate (WR) for the variable $\dot{V}O_2$. Values expressed as liters O_2 per minute.

WR	1	2	3	4	5	6
Air	1.46	2.11	2.50	2.78	3.27	3.57
He- O_2	1.45	2.08	2.59	2.81	3.40	3.75

Table 3. Repeated measures summary table of combined data in Sets 1 and 2 for $\dot{V}O_2$.

Source	DF	Sum of Squares	F	Prob F
Subjects	3	5.013		
Treatment	1	0.051	6.760	.080
Treatment error	3	0.022		
Work rate (WR)	5	26.127	58.450	<.001
WR error	15	1.341		
Treatment $\times$ WR	5	0.066	2.400	.087
Treatment $\times$ WR error	15	0.083		
Total	47	32.703		

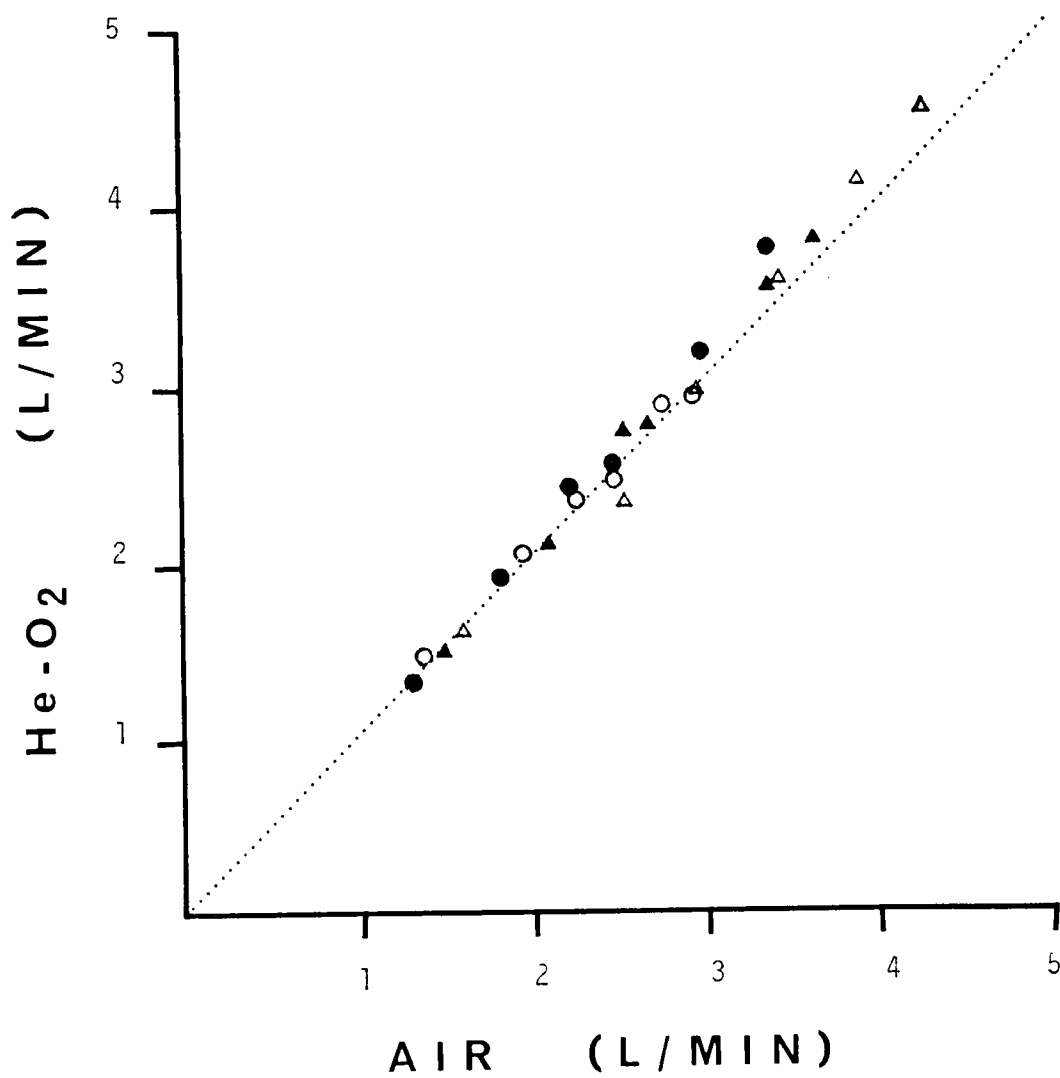


Figure 1. Plot of $\dot{V}O_2$ on He-O₂ compared with that on air. Dotted line represents the line of identity.

Table 4. Repeated measures summary table for $\dot{V}O_2$.

Source	DF	Sum of Squares	F	Prob F
Gas	1	0.0034	2.49	.132
Error	18	0.0244		
Total	19	0.0278		

Table 5. Combined mean values for all subjects at each work rate (WR) for the variable lactate. Values expressed as mmol per liter.

WR	1	2	3	4	5	6
Air	1.2	1.7	3.4	4.3	5.1	12.2
He-O ₂	1.1	1.9	3.8	4.9	5.7	12.1

Table 6. Repeated measures summary table of combined data in Sets 1 and 2 for lactate.

Source	DF	Sum of Squares	F	Prob F
Subjects	3	20.058		
Treatment	1	0.921	0.825	.431
Treatment error	3	3.349		
Work rate (WR)	5	620.560	42.690	<.001
WR error	15	43.608		
Treatment $\times$ WR	5	0.969	0.398	.843
Treatment $\times$ WR error	15	7.314		
Total	47	696.779		

($p > .10$) between the two experimental treatments. No significant interaction between gas and work rate was found for lactate ($p > .10$). However, despite the fact that no statistically significant difference was found due to the experimental treatment, the lactate values were higher during the He- O_2 treatment in 17 out of 24 experiments. This is shown in Figure 2.

In Set 3, the mean lactate value during air breathing was 3.2 mmol per liter and that for He- O_2 was 3.7 mmol per liter. The differences in lactate values between the gas treatments were not statistically significant with the level of significance being calculated at $p > .10$ (see Table 7).

Minute Ventilation

There was a highly significant ($p < .001$) increase in minute ventilation in response to an increase in work rate during the experiments of Sets 1 and 2 (see Table 8). When He- O_2 was the inspired gas mixture, minute ventilation was significantly larger ($p < .01$) than the ventilation occurring when air was inspired. The combined mean data on all subjects for the variable minute ventilation are listed in Table 9.

A highly significant interaction ($p < .001$) occurred between gas and work rate. When one inspects Table 9 and Figure 3, it appears that there are essentially no differences in minute ventilation between the gases until the intense exercise of work rates 5 and 6. At these levels minute ventilation values for He- O_2 become much larger than those of air and could possibly account for the overall significance found for the treatment and interaction. In an attempt to explain this interaction,

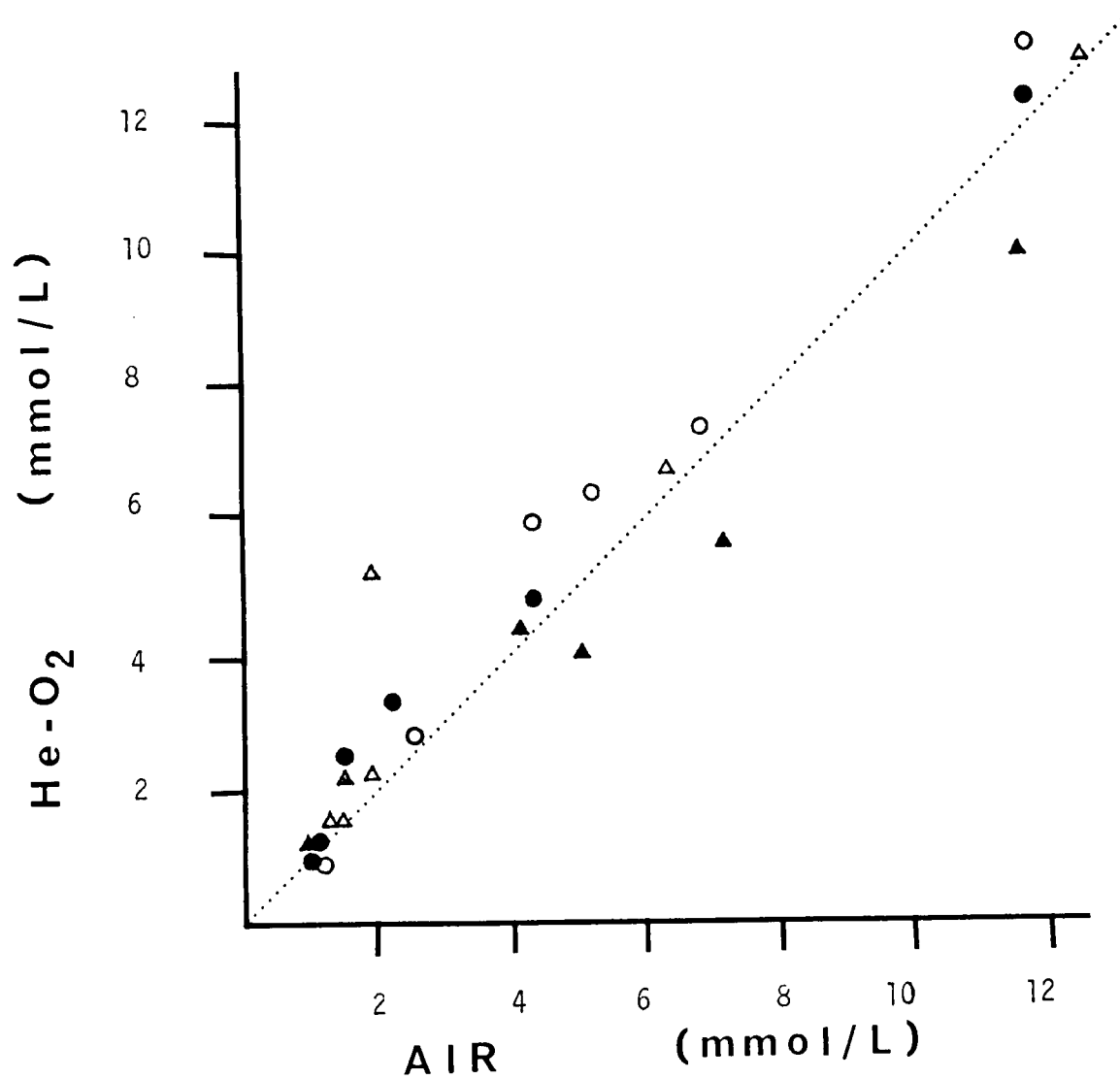


Figure 2. Plot of lactate concentration on He-O₂ compared with that on air. Dotted line represents the line of identity.

Table 7. Repeated measures summary table for lactate.

Source	DF	Sum of Squares	F	Prob F
Gas	1	0.5513	1.88	.219
Error	6	1.7575		
Total	7	2.3088		

Table 8. Repeated measures summary table of combined data in Sets 1 and 2 for minute ventilation.

Source	DF	Sum of Squares	F	Prob F
Subjects	3	19290.727		
Treatment	1	667.520	31.18	.010
Treatment error	3	64.230		
Work rate (WR)	5	93638.690	35.35	<.001
WR error	15	7947.393		
Treatment $\times$ WR	5	2157.850	20.69	<.001
Treatment $\times$ WR error	15	312.900		
Total	47	124079.310		

Table 9. Combined mean values for all subjects at each work rate (WR) for the variable minute ventilation. Values expressed as liters per minute (BTPS).

WR	1	2	3	4	5	6
Air	36	54	70	89	119	149
He-O ₂	36	52	71	89	128	185

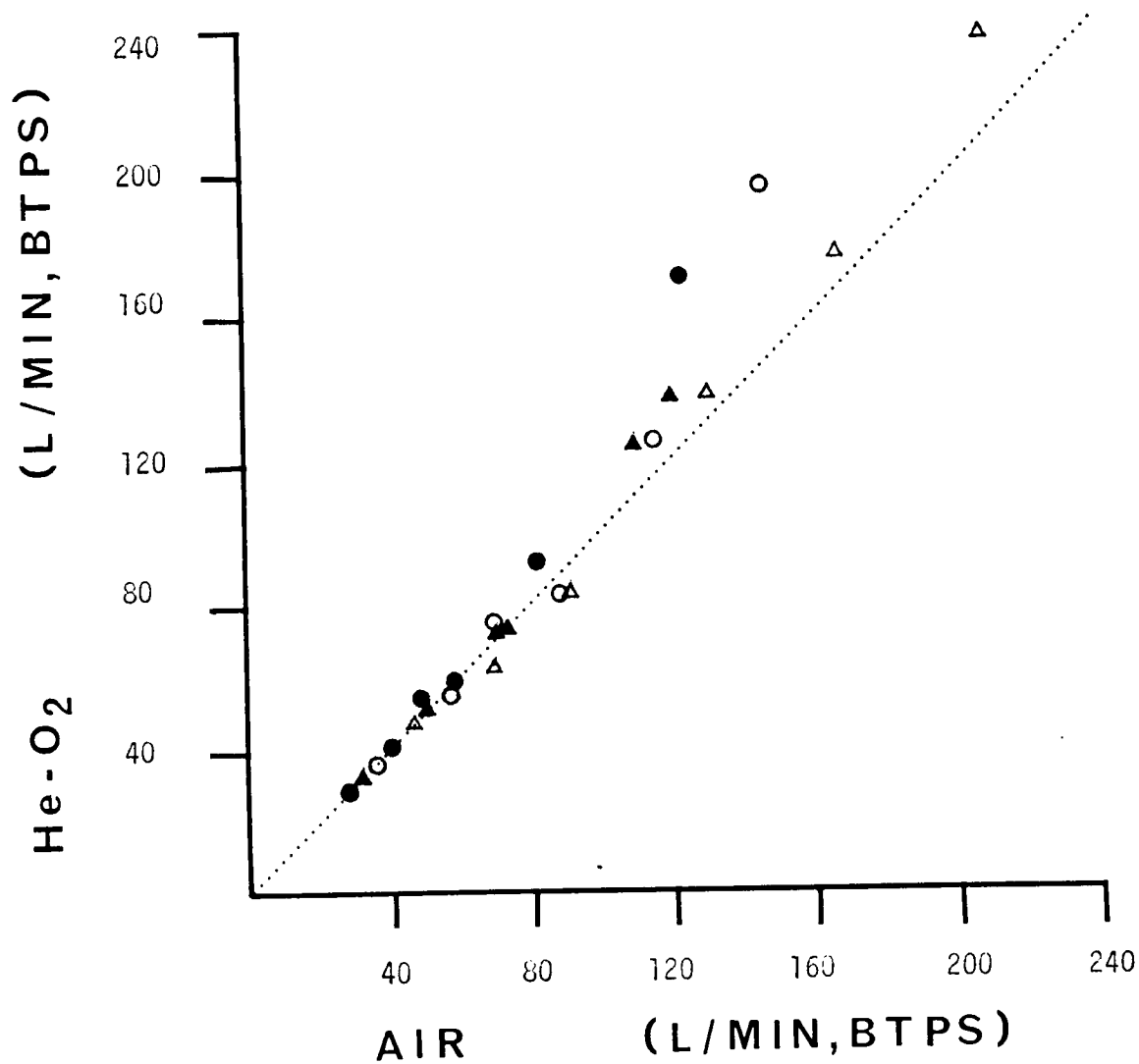


Figure 3. Plot of minute ventilation on He-O₂ compared with that on air. Dotted line represents the line of identity.

a 2-way ANOVA was utilized to test for significant differences in minute ventilation between the gases at each of the six work rates. There was no significant difference in minute ventilation between air and He-O₂ for work rates 1, 2, 3, or 4. Significant differences in ventilation between the breathing mixtures were found for work rate 5 ($p < .01$) and work rate 6 ($p < .01$) with He-O₂ giving the larger values. Figure 3 presents the ventilation when breathing He-O₂ plotted against the ventilation when inspiring air for all subjects in the experiments of Sets 1 and 2.

In the tests of Set 3, a significant difference ($p < .001$) occurred in minute ventilation between the gas mixtures (see Table 10). The mean value during air breathing was 63 liters per minute and that of He-O₂ was 67 liters per minute.

Heart Rate

The combined mean data from Sets 1 and 2 on all subjects for the variable HR are listed in Table 11. The repeated measures summary table for the variable HR is presented in Table 12. Highly significant ($p < .001$) increases in HR occurred in response to increases in the intensity of exercise. There were no statistically significant differences ($p > .10$) in HR attributable to the experimental treatment. The interaction between gas and work rate was found not to be significant ($p > .10$). Figure 4 presents the HR when breathing He-O₂ plotted against the HR when inspiring air for all subjects in the experiments of Sets 1 and 2.

Table 10. Repeated measures summary table for minute ventilation.

Source	DF	Sum of Squares	F	Prob F
Gas	1	85.41	14.88	.001
Error	18	103.31		
Total	19	188.72		

Table 11. Combined mean values for all subjects at each work rate (WR) for the variable HR. Values expressed as beats per minute.

WR	1	2	3	4	5	6
Air	115	134	163	163	184	179
He-O ₂	116	137	159	164	181	181

Table 12. Repeated measures summary table of combined data in Sets 1 and 2 for heart rate.

Source	DF	Sum of Squares	F	Prob F
Subjects	3	2708.063		
Treatment	1	1.688	0.33	.605
Treatment error	3	15.228		
Work rate (WR)	5	26993.855	106.68	<.001
WR error	15	759.062		
Treatment $\times$ WR	5	78.687	1.28	.322
Treatment $\times$ WR error	15	183.900		
Total	47	30740.483		

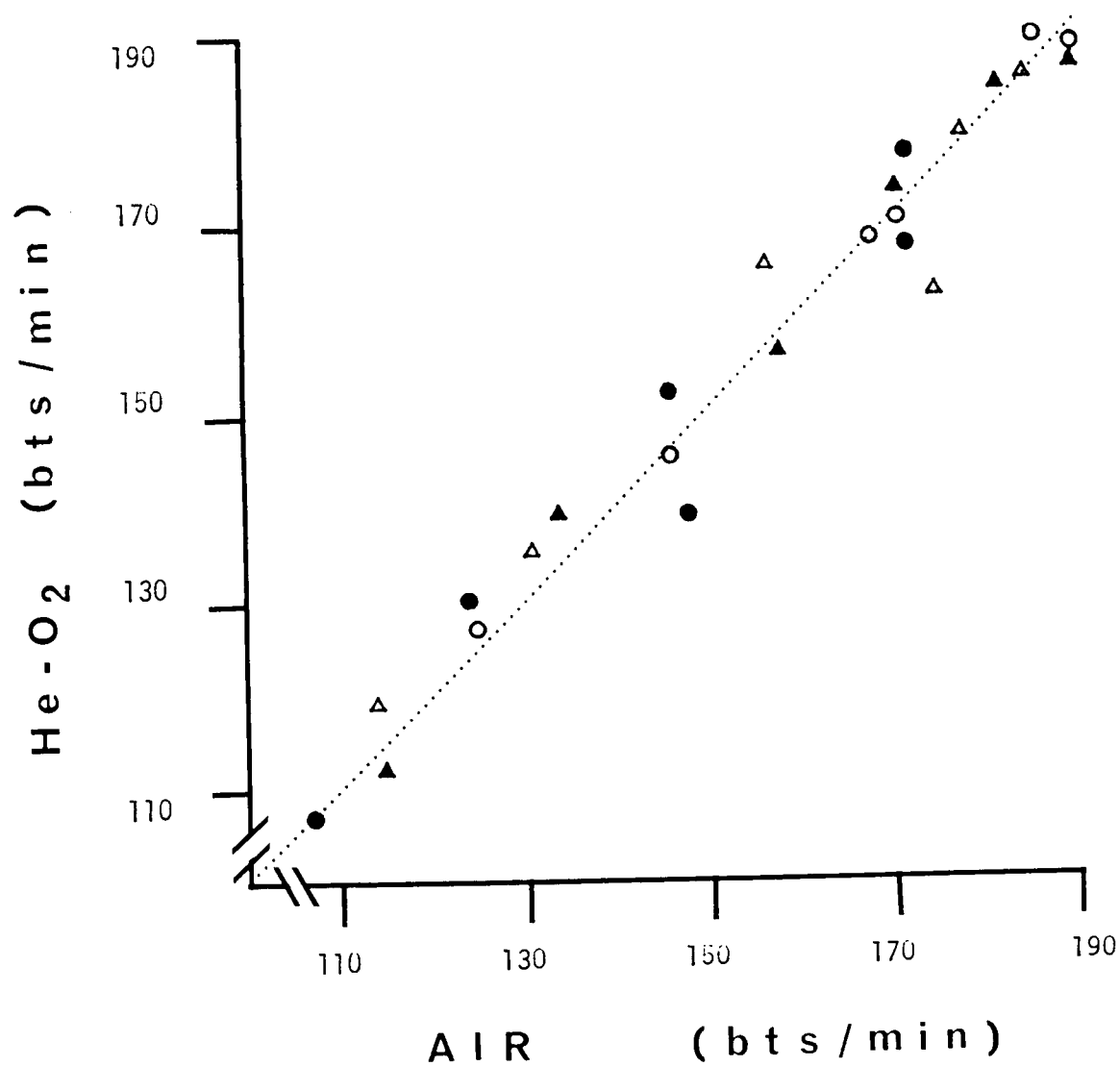


Figure 4. Plot of heart rate on He-O₂ compared with that on air. Dotted line represents the line of identity.

CHAPTER V

DISCUSSION

The discussion will be divided into a separate section for each of the four measured variables. Overall, only minute ventilation demonstrated a statistically significant difference between the gases when tested at the .05 level of significance. However, the choice of the statistical analysis resulted in ambiguous and marginally significant differences between the gases for the variable $\dot{V}O_2$.

Although the differences in $\dot{V}O_2$ between the gas mixtures were not statistically significant, there appeared to be a definite tendency towards higher $\dot{V}O_2$ values on He- O_2 at the high work rates. If one reexamines Figure 1 (page 34) and Table 2 (page 32) this should be apparent. The calculated probability of the F-ratio in this case was $p = .08$ for the treatment so this approaches significance at the .05 level. The same is true for interaction. These results are in contrast to those reporting a lower $\dot{V}O_2$ on He- O_2 (23, 32, 55) or no difference in $\dot{V}O_2$ (3, 11, 41). The trends in the present study are too apparent to ignore despite the fact that they are not statistically significant. Some possible explanations for these results will be presented in the section on oxygen uptake.

The results of the statistical analysis of the lactate data were also ambiguous. The differences between the gases were not significant but there were trends too apparent to ignore. These will be discussed in greater detail in the section on blood lactate.

Oxygen Uptake

Spitler et al. (55), Murphy et al. (32), and Hoar et al. (23) reported that the $\dot{V}O_2$ is lower when breathing He-O₂. These investigators attributed this lowered $\dot{V}O_2$ to a decreased work of breathing when inspiring the less dense He-O₂ mixture. Tait (56) found $\dot{V}O_2$ values that were nonsignificantly higher on He-O₂, but as mentioned earlier, only 4 subjects were used. Raymond et al. (41), Bowers and Fox (3), and Fox et al. (11) reported no differences in $\dot{V}O_2$ between the gas mixtures, but the work intensities employed in these studies were mild, whereas in the present study it was the more intense levels of exercise where the greatest differences in $\dot{V}O_2$ occurred.

An attempt must be made to explain the conflict between the results of the present study in which $\dot{V}O_2$ was higher on He-O₂ and the results of investigations in which $\dot{V}O_2$ was purported to be lower on He-O₂ than on air (23, 32, 55). There are several places in the present study where small errors could have resulted in higher $\dot{V}O_2$ values for He-O₂. If the calibration of the resistance setting on the bicycle ergometer were to shift upward between the performance of an air test and a He-O₂ test, the result would be that the subject would be exercising at a higher work rate, with its concomitant higher O₂ requirement. This is not likely the case in the present experiments as the calibration of the ergometer was checked daily. Likewise, the pedal frequency and resistance settings were monitored during the course of each experiment to ensure consistency between tests.

If ventilation were somehow measured erroneously high for He-O₂, a higher calculated $\dot{V}O_2$ could result. Once again, this explanation is not

a likely one for the current study. The Parkinson-Cowan gas meter was calibrated before tests began with both air and He-O₂ with no differences in calibration. In the present study, two different set-ups were used to deliver gas to the subject and to measure its volume. In one, the gas meter was not in the delivery system so that any added resistance to gas flow the gas meter might add would not be present. In the second, gas passed through the meter before reaching the subject. Regardless of the method employed, measured ventilation seemed not to be affected. It is concluded that the methods of gas delivery and measurement utilized in this study did not result in falsely high ventilation values for He-O₂.

The gas samples collected in these experiments were analyzed for inspired and expired O₂ fractions by an electronic analyzer. If the He-O₂ mixture possessed some property that resulted in the analyzer giving higher FIO₂ values or lower FEO₂ values than what actually existed, the calculated $\dot{V}O_2$ would be erroneously high. This possibility is also not a likely explanation for the higher $\dot{V}O_2$ values on He-O₂. As discussed in the Methods chapter, He-O₂ mixtures analyzed by the Scholander method and the electronic method agreed to within several one-hundredths of 1% so it appears that the electronic analyzer was giving true values for the He-O₂ samples.

It is difficult to find a reason for the higher $\dot{V}O_2$ values that could have resulted from an experimental error. The next logical step to explain the conflicting results is to examine more closely the investigations which reported in lower $\dot{V}O_2$ values on He-O₂ to see if an answer can be found there.

Just as an overestimation of ventilation on He-O₂ could give erroneously high calculated $\dot{V}O_2$ values, an underestimation of ventilation during He-O₂ breathing could result in $\dot{V}O_2$ values that are below the true ones. Wilson and Welch (62) reported that on the average, ventilation during maximal work was 33 l/min greater when He-O₂ was the inspired mixture. In the present study, it can be seen from Table 9 (page 42) that the He-O₂ mixture resulted in average ventilations that were 36 l/min higher during maximal work. Spitler et al. (55) reported that during maximal exercise ventilation on He-O₂ was only 16 l/min higher than that of air. These investigators employed a pneumotachograph to measure ventilation instead of a dry gas meter. It may be possible that the pneumotachograph underestimates ventilation when He-O₂ is inspired.

Further support for this suspicion comes from maximal voluntary ventilation (MVV) values for air and He-O₂ breathing. Tenney and Reese (57) reported that the combined mean MVV values for 5 subjects inspiring He-O₂ were 60 l/min greater than those measured during air breathing. Similar results were noted in experiments conducted in this laboratory. The MVV values of Spitler et al. (55) were only 6 l/min higher on He-O₂ than on air; this seems low in light of the previously mentioned findings. Although no definite statements about the accuracy of the ventilation measurements of Spitler et al. can be made at this time, it seems reasonable to suggest that their system may indeed underestimate ventilation on He-O₂. If so, this could account for their low calculated values for $\dot{V}O_2$.

Murphy et al. (32) reported a lower $\dot{V}O_2$ when breathing He-O₂ at all work rates and they found that the ventilations were essentially identical between the breathing mixtures at all work rates. In my study, the ventilation at which He-O₂ values began to greatly exceed those of air was approximately 100 l/min. It is also at these ventilations where the largest differences in $\dot{V}O_2$ between the gases occurred. When the ventilation data of Murphy et al. are closely examined, it can be seen that out of 56 measurements, only 3 ventilation values are above 100 l/min. It may be that the ventilations of their subjects did not reach the "breaking point;" that is, the ventilation at which the He-O₂ values begin to greatly exceed those of air. If this be the case, the subjects in the study of Murphy et al. did not reach the ventilation levels where the greatest differences in $\dot{V}O_2$ occurred in my study.

The same line of reasoning can also be applied to the finding of Hoar et al. (23) that the $\dot{V}O_2$ was lower on He-O₂. The average ventilation of their subjects was approximately 50 l/min. This is much below the ventilation values required to elicit the higher $\dot{V}O_2$ when breathing He-O₂ as found in the present study.

If there is indeed a real increase in $\dot{V}O_2$ when He-O₂ is the inspired gas mixture, what could be a possible mechanism for this increase? One mechanism that is not a likely candidate is that of an increase in metabolism in response to an increased heat loss during He-O₂ inhalation. As discussed in the literature review, the increased $\dot{V}O_2$ in small mammals exposed to a He-O₂ environment is believed to be a homeostatic response

to the increased heat loss which occurs in the He-O₂ environment. In humans this is not believed to be the case. Bowers and Fox (3) found that a 2-hour exposure to He-O₂ did not produce a sufficient fall in body temperature to initiate an increase in metabolism. Other investigators are also of the opinion that exposure of man to He-O₂ environments at comfortable temperatures will not result in a heat loss great enough to stimulate metabolism (3, 11, 23, 27, 43, 55).

Hoar et al. (23) found that the fall in rectal temperature during work in 25° C water was the same whether air or He-O₂ was breathed. They concluded that He does not "accentuate respiratory convective heat transfer." This can be explained by considering the physical properties of air and He-O₂. Even though He-O₂ has a thermal conductivity that is 6 times that of air, the product of density and specific heat is less for He-O₂ than for air (23). This means that the difference in respiratory tract heat loss breathing He-O₂ or air should be very small.

During the present study, He was limited to the respiratory tract, as it was in the investigation of Hoar et al. (23). In view of the findings that no increase in metabolism occurred when He-O₂ was the ambient atmosphere (3, 11) and that He was exposed only to the respiratory tract in the current study, it appears likely that the small increases in $\dot{V}O_2$ found in this study cannot be attributed to an increased loss of body heat through the respiratory tract.

It is believed by some that N₂ acts as a metabolic depressant and that if this depressant effect is removed by substituting N₂ with a less

narcotic gas such as He, metabolism could be increased. Jordan et al. (24), when investigating the metabolic effects of N_2 as a diluent environmental gas, concluded that even at a pressure of one atmosphere the N_2 in the air has a depressing effect metabolically and that this can be relieved by replacing the N_2 with a less narcotic diluent gas. One belief is that the molecular weight is the prime factor influencing the tendency towards narcosis (24). There is an inverse relationship between the molecular weight of the diluent gas and the partial pressure required to effect comparable symptoms of narcosis (24). Therefore, since N_2 has a higher molecular weight than He, it is theorized that N_2 should be more narcotic than He. The results of the present study cannot be offered in support of this theory. If N_2 was indeed narcotic, one would expect to see increases in $\dot{V}O_2$ at all work rates when He was substituted for N_2 . In my study this was not the case. At the lower work rates the $\dot{V}O_2$ was slightly higher when breathing air. It was only at the higher work rates that the $\dot{V}O_2$ values during He- O_2 breathing were higher.

Maio and Neville (30) reported reductions in O_2 consumption by yeast and rat liver slices in the presence of N_2 and suggested that N_2 may affect O_2 transport into the cell. Galvin et al. (13) found that N_2 at .8 atmosphere pressure could exert a braking influence on metabolic processes. They attributed this to N_2 's higher solubility in lipid compared to that of He. The data from my study cannot support these theories for the same reasons as described above, unless N_2 's supposed

depressant action is most effective during high metabolic rates such as those which would accompany high intensity exercise.

In the literature review mention was made of studies in which the $\dot{V}O_2$ was increased when He was substituted for N_2 (6, 7, 8, 13, 43, 46, 49, 51, 53). Experiments by South and Cook (53) indicated that tissue $\dot{V}O_2$ was stimulated by He rather than inhibited by N_2 . The results from the present study could be interpreted to indicate that He stimulated metabolism relative to N_2 , but only at the high work rates. A suggested mechanism for this stimulation is that of a direct gas interaction with cellular enzymes (49). The association of proteins with inert gases has been documented (51). Schreiner states that the "observed changes in enzymic activity may be the result of structural alterations in the enzyme molecules brought about by interaction with the He-group gases." Another possibility is that the inert gases have differential effects on cell and mitochondrial membranes which influence O_2 transport across membranes (49).

The greatest differences between the gases occurred at the most intense work rates where ventilation values were much larger on He- O_2 than they were for air. The increased $\dot{V}O_2$ on He- O_2 could be attributed to an increased O_2 cost of breathing since the respiratory muscles are moving a larger volume of gas when He- O_2 is breathed. This is probably not the case. Even though a greater volume of He- O_2 was moved per minute than air, Wilson and Welch (62) have calculated that much less mass was moved because of density differences between the gases. It would seem then, that the respiratory muscles would be doing less work.

Murphy et al. (32), Hoar et al. (23), and Spitler et al. (55) stated that the $\dot{V}O_2$ on He- O_2 should be lower, not higher, because the less dense He- O_2 mixture will decrease the amount of turbulent flow in the airways with a concomitant reduction in the muscular work required for a given ventilation.

Once again, it must be strongly emphasized that the increases in $\dot{V}O_2$ while inspiring He- O_2 noted in my study were not statistically significant. However, in light of the conflicting results of others (23, 32, 55), an attempt must be made to explain these nonsignificant increases. The mechanism described below is being offered to explain the results of the present study. This mechanism can only be suggested at the present time; accurate measurements of blood gas tensions and pH will be required in future studies for verification.

Riggs et al. (44) are of the opinion that alkalotic states could result in tissue hypoxia due to the increased oxyhemoglobin affinity that results during alkalosis. This viewpoint is challenged, however, by reports that $\dot{V}O_2$ can be increased during states of respiratory alkalosis (5, 25, 44). Riggs et al. (44), using anesthetized Rhesus monkeys found large increases in $\dot{V}O_2$ during states of respiratory alkalosis which were induced by passive hyperventilation. Cain (5), by passively hyperventilating dogs, determined that $\dot{V}O_2$ was dependent on the pH rather than PCO_2 . As the dogs were hyperventilated, $\dot{V}O_2$ increased as pH rose and PCO_2 fell. The dogs were then infused with HCl. The $\dot{V}O_2$ decreased to control levels as pH returned to normal but PCO_2 stayed at its low levels. Cain concluded that the hydrogen ion

concentration was the factor setting $\dot{V}O_2$ in these dogs. Karetzky and Cain (25), when investigating the effect of CO_2 on O_2 uptake during hyperventilation in man, reported that a fall in hydrogen ion concentration stimulated total body $\dot{V}O_2$.

Since measurements of blood pH and PCO_2 were not made during the present study, it cannot be stated with certainty whether differences in pH and PCO_2 existed in the blood between the two gas mixtures. However, large increases in ventilation while inspiring He- O_2 relative to air breathing were noted (see Table 9, page 42) during the intense work rates. Because of these large differences in ventilation, would it not be reasonable to postulate that this hyperventilation on He- O_2 would result in a lower hydrogen ion concentration via respiratory compensation? If so, the subject would be more alkalotic relative to his condition when breathing air. The increased $\dot{V}O_2$ values found in this study could be associated with this alkalotic state and would be in agreement with the reports discussed previously (5, 25, 44).

Blood Lactate

As was the case for $\dot{V}O_2$, there were no significant differences in blood lactate concentration between the treatments. There seemed to exist however, a trend towards higher lactate values during He- O_2 breathing. This can be seen by examining Table 6 (page 37). Also, Figure 2 (page 39) shows that in 17 out of 24 experiments the lactate values were higher when the subject had been inspiring He- O_2 . In fact, a sign test demonstrated significant differences in lactate between the gases. In Set 3, lactate values were higher during He- O_2

breathing 3 out of 4 times. As was pointed out in the literature review, lactate values during He-O₂ breathing while exercising have not been previously reported. Only further experimentation can answer this question but it is interesting to speculate on what could be responsible for the apparent trend towards higher lactate concentrations while breathing He-O₂ as found in this study.

As was discussed in the section on oxygen uptake, pedal frequency and resistance settings were carefully monitored during all experiments so it is unlikely that a subject was exercising at a higher work rate during a He-O₂ test than an air test. Garcia et al. (14) reported that alkalemia can cause a lactate increase through a stimulation of glycolysis by pH sensitive enzymes. In the present study, alkalemic states attributable to respiratory compensation may have existed during work rates 5 and 6 as there were large increases in ventilation when breathing He-O₂. When one looks at Table 5 (page 36) and Table 9 (page 42) together, it can be seen that for work rates 2, 3, 4 and 5 the lactate concentration is approximately 12% higher during He-O₂ inhalation for each of these four work rates even though there are essentially no differences in ventilation between gases for work rates 2, 3, and 4. Even though an alkalemic state may have existed when breathing He-O₂ in work rate 5 to explain the increase in lactate concentration, such an explanation for the increase in lactate while breathing He in work rates 2, 3 and 4 is not possible since no respiratory compensation is likely to be present during these work rates.

Another possibility is that He stimulates one or more of the glycolytic enzymes. South and Cook (53) reported that He accelerated

glycolysis between the aldolase and enolase reactions. The nonsignificant increases in lactate due to treatment found in this study could be attributed to a possible stimulatory effect by He on glycolysis. Another remote possibility that should be mentioned is related to the treatment of the blood samples. If it will be recalled from the Methods section, blood samples were treated with NaF immediately after having been drawn. The NaF inhibits the enzyme enolase thereby halting glycolysis. South and Cook (53) found that He alleviates this inhibition of enolase by NaF. So, if any He remained dissolved in the plasma or cytoplasm of the red blood cells, this residual He may alleviate the NaF inhibition of enolase thereby permitting glycolysis to continue in the spot plate. This would result in higher blood lactate values than what actually existed in the blood within the subject.

The question of a He effect on metabolism remains open and more work is needed to clarify whether there is an effect due to He and if so, what is the mechanism?

Minute Ventilation

The data from the present study are in agreement with most of the previously reported results. The finding of essentially no difference in ventilation between the gases at the lower work rates is in agreement with numerous other investigations (3, 11, 32, 41, 56). Spitler et al. (55) and Hoar et al. (23) reported nonsignificantly higher values for He-O₂ during low intensity exercise. The finding of large differences in ventilation between the gases at high work rates has also been previously reported (55, 56, 62). Spitler et al. (55) found that

ventilation on He-O₂ became significantly greater than that on air only during exercise levels requiring at least 85% of $\dot{V}O_2$ max. In the present study, exercise levels greater than 70-75% of $\dot{V}O_2$ max were required to elicit significant differences in minute ventilation between the gases. In Set 3, minute ventilation was significantly greater than He-O₂ breathing when the subject exercised at approximately 60% of $\dot{V}O_2$ max. Regardless of the exact level of exercise required to show significant differences in minute ventilation, it seems clear that ventilation on He-O₂ is similar to that of air at low work rates but that the ventilation values become progressively higher at the more intense work rates. This is clearly demonstrated by Table 9 (page 42) and Figure 3 (page 43).

What is the reason for the large increase in ventilation at the high work rates when inspiring He-O₂? To answer this question one must consider the physical properties and flow characteristics of the He-O₂ mixture relative to those of air. At high flow rates of gas through the respiratory airways, resistance to this flow becomes greater as turbulence develops. This turbulence and resistance to flow will be less for a He-O₂ mixture because of its low density (56). A mixture such as He-O₂ that is less dense than air can increase total flow through the lungs by decreasing resistance and resistance to turbulent flow (55). This would be apparent only at high flow rates because during low flow rates, turbulent flow would not be great enough to influence total gas flow through the lung airways (32). The data of the present study and others support this concept. At the low and moderate work rates, flow through

the airways remains laminar for both air and He-O₂. As ventilation increases, however, flow becomes turbulent for air before that of He-O₂ so the rate of flow for the He mixture is greater, resulting in larger minute ventilations.

Changes in breathing frequency could also contribute to the higher ventilations during He-O₂ breathing at high work rates. During the high intensity exercise of Set 2, the average breathing frequency was 4 breaths per minute higher during He-O₂ breathing. There are other reports of higher breathing frequencies during He-O₂ inhalation (55, 56, 62). Tait (56) accounts for the higher frequency in the following way. Because of the density and flow characteristics of a He-O₂ mixture, the lungs fill more quickly than they would for air. This will result in a quicker input of inhibitory signals from the lung's stretch receptors to the "inspiratory control center." This then initiates expiration with the same process occurring in reverse. The net result is that the quicker filling and emptying of the lungs with He-O₂ causes an increased respiratory rate and a concomitant increase in ventilation relative to air.

Heart Rate

The significant increase in HR which occurred in response to increasing work rate was not surprising, as rises in HR with increasing work rate have long been known. The finding of no significant differences in HR between the two gas mixtures is in accord with the results of studies utilizing exercising human subjects (11, 23, 41, 55, 56), resting human subjects (3), anesthetized dogs (28), and both pithed and

unanesthetized rats (34). From the results of the present investigation and those aforementioned, it appears that the inspiration of a normoxic He gas mixture does not exert a detectable influence on cardiac frequency. This suggests that He does not exert an effect on metabolism because it seems that a He-induced change in metabolism would also be reflected by a change in HR.

REFERENCES

REFERENCES

1. Bartlett, R. G., Jr. and H. Specht. Energy cost of breathing determined with simplified technique. J. Appl. Physiol. 11(1): 84-86, 1957.
2. Boerboom, L. E., and J. N. Boelkins. Hemodynamics in awake rabbits during hyperbaric helium-oxygen exposure. J. Appl. Physiol. 48(2): 281-283, 1980.
3. Bowers, R. W., and E. L. Fox. Metabolic and thermal responses of man in various He-O₂ and air environments. J. Appl. Physiol. 23(4): 561-565, 1967.
4. Bradley, M. E., and D. E. Leith. Ventilatory muscle training and the oxygen cost of sustained hypernea. J. Appl. Physiol. 45(6): 885-892, 1978.
5. Cain, S. M. Increased oxygen uptake with passive hyperventilation of dogs. J. Appl. Physiol. 28(1): 4-7, 1970.
6. Cook, S. F. The effect of helium and argon on metabolism and metamorphosis. J. Cellular Comp. Physiol. 36: 115-127, 1950.
7. Cook, S. F., and F. E. South, Jr. Helium and comparative in vitro metabolism of mouse-tissue slices. Amer. J. Physiol. 173: 542-544, 1953.
8. Cook, S. F., F. E. South, Jr., and D. R. Young. Effect of helium on gas exchange of mice. Amer. J. Physiol. 164: 248-250, 1951.
9. DeVries, H. A. Physiology of Exercise for Physical Education and Athletics. Second ed. Dubuque: Wm. C. Brown Publishers, 1974.
10. Fagraeus, L. Maximal work performance at raised air and helium-oxygen pressures. Acta physiol. scand. 91: 545-556, 1974.
11. Fox, E. L. H. S. Weiss, R. L. Bartels, and E. P. Hiatt. Thermal responses of man during rest and exercise in a helium-oxygen environment. Arch. Environ. Health. 13: 23-28, 1966.
12. Frankel, J., and H. A. Schneiderman. The effects of nitrogen, helium, argon and sulfur hexafluoride on the development of insects. J. Cellular Comp. Physiol. 52: 431-451, 1958.

13. Galvin, R. D., D. J. Peeler, and G. A. Albright. Effects of various inert gas diluents on oxygen consumption at normo-hypo-and-hyperbaric pressures. Aerospace Med. 37: 278, 1966.
14. Garcia, A. C., Y. L. Lai, B. A. Atteberg, and E. B. Brown, Jr. Lactate and pyruvate accumulation during hypocapnia. Respiration Physiol. 12: 371-380, 1971.
15. Glauser, S. C., E. M. Glauser, and B. F. Rusy. Gas density and the work of breathing. Respiration Physiol. 2: 344-350, 1967.
16. Gledhill, N., A. K. Chin, and A. C. Bryan. Plasma catecholamines in humans during helium breathing (Abstract). Physiologist. 18: 227, 1975.
17. Guénard, H., M. Chaussain, and C. Lebeau. Respiratory gas exchange under normobaric helium-oxygen breathing at rest and during muscular exercise. Bull. europ. Physiopath. resp., 14: 417-429, 1978.
18. Gutmann, I., and A. W. Wahlefeld. L-(+)-Lactate determination with lactate dehydrogenase and NAD. In: Methods of enzymatic analysis. Edited by H. U. Bergmeyer. 2nd English Ed. New York: Academic Press, Inc., pp. 1464-1468, 1974.
19. Hamilton, R. W., J. O. Cohen, G. F. Doebbler, L. F. Exposito, J. M. King, K. H. Smith, and H. R. Schreiner. Biochemical and metabolic effects of a six-month exposure of small animals to a helium-oxygen atmosphere. Space Life Sci. 2: 57-99, 1970.
20. Hamilton, R. W., G. F. Doebbler, and H. R. Schreiner. Biological evaluation of various space cabin atmospheres, I. Space Life Sci. 2: 307-334, 1970.
21. Hargreaves, J. J., W. G. Robertson, F. Ulvedal, H. J. Zeff, and B. E. Welch. Study of man during a 56-day exposure to an oxygen-helium atmosphere at 258 mm. Hg total pressure, I. Introduction and general experimental design. Aerospace Med. 37: 552-554, 1966.
22. Hawkins, I. K. Helium effect on skeletal muscle of mice. Aerospace Med. 44(4): 374-378, 1973.
23. Hoar, P. F., L. W. Raymond, H. C. Langworthy, R. E. Johnsonbaugh, and J. Sode. Physiological responses of men working in 25.5° C water, breathing air or helium tri-mix. J. Appl. Physiol. 40(4): 605-610, 1976.
24. Jordan, J. P., J. B. Simmons, D. V. Lassiter, Y. Deshpande, and D. J. Dierschke. Metabolic effects of nitrogen and neon as diluent

- environmental gases. In: Depressed Metabolism: Proceedings of the International Conference on Depressed Metabolism. Eds. X. Musacchia and J. F. Saunders. New York: American Elsevier Publishing Company, 1969.
25. Karetzky, M. S., and S. M. Cain. Effect of carbon dioxide on oxygen uptake during hyperventilation in normal man. J. Appl. Physiol. 28(1): 8-12, 1970.
 26. Latterell, R. L. Nitrogen and helium-induced anoxia: different lethal effects on rye seeds. Science. 153: 69-70, 1966.
 27. Leon, H. A., and S. F. Cook. A mechanism by which helium increases metabolism in small mammals. Am. J. Physiol. 199(2): 243-245, 1960.
 28. Leon, F. A., and W. S. Harris. Prolonged elevation of the ventricular fibrillation threshold by helium. Circulation. 48(Suppl. 4): IV-127, 1973.
 29. Lin, Y. C., and E. Kato. Effects of helium gas on heart rate and oxygen consumption in anesthetized rats. Undersea Biomed. Res. 1: 281-290, 1974.
 30. Maio, D. A., and J. R. Neville. Effect of chemically-inert gases on oxygen consumption in living tissues. Aerospace Med. 38: 1049-1056, 1967.
 31. McKerrow, C. B., and A. B. Otis. Oxygen cost of hyperventilation. J. Appl. Physiol. 11(1): 375-379, 1956.
 32. Murphy, T. M., W. H. Clark, I. P. B. Buckingham, and W. A. Young. Respiratory gas exchange in exercise during helium-oxygen breathing. J. Appl. Physiol. 26(3): 303-307, 1969.
 33. Nebergall, W. H., F. C. Schmidt, and H. F. Holtzclaw, Jr. General Chemistry. Third ed. Lexington: D. C. Heath and Co., p. 77, 1968.
 34. Nicholas, T. E., J. L. Hart, and P. A. Kim. Effect of helium breathing on sympathetic nervous and cardiovascular functions. Undersea Biomed. Res. 1: 271-280, 1974.
 35. Otis, A. B. The work of breathing. Physiol. Rev. 34: 449-457, 1954.
 36. Otis, A. B. Quantitative relationships in steady state gas exchange. In: Handbook of Physiology—Respiration, Vol. I. (Ed. W. O. Fenn and H. Rahn) Washington, D. C.: Amer. Physiol. Soc., 1964.

37. Ouellet, Y., S. C. Poh, and M. R. Becklake. Circulatory factors limiting maximal aerobic exercise capacity. J. Appl. Physiol. 27(6): 874-820, 1969.
38. Pifarré, R., W. D. Cox, M. Jasuja, and W. E. Neville. Helium in the prevention of ventricular fibrillation. Dis. Chest. 56: 135-138, 1969.
39. Pifarré, R., T. K. Paghunath, R. M. Vanecko, F. S. Chua, J. U. Balis, and W. E. Neville. Effect of oxygen and helium mixtures on ventricular fibrillation. J. Thorac. Cardiovasc. Surg. 60: 648-652, 1970.
40. Raymond, L. W., W. H. Bell, K. R. Bandi, and C. R. Lindberg. Body temperature and metabolism in hyperbaric helium atmospheres. J. Appl. Physiol. 24(5): 678-684, 1968.
41. Raymond, L. W., J. Sode, H. C. Langworthy, J. Blosser, and R. E. Johnsonbaugh. Increase in plasma growth hormone concentration: a new effect of helium breathing. Undersea Biomed. Res. 1: 91-98, 1974.
42. Raymond, L. R. B. Weiskopf, M. L. Halsey, A. Goldfein, E. I. Eger, and J. W. Severinghaus. Possible mechanism for the antiarrhythmic effect of helium in anesthetized dogs. Science. 176: 1250-1252, 1972.
43. Rhoades, R. A., R. A. Wright, E. P. Hiatt, and H. S. Weiss. Metabolic and thermal responses of the rat to a helium-oxygen environment. Am. J. Physiol. 213(4): 1009-1014, 1967.
44. Riggs, T. E., A. W. Shaffer, and C. A. Guenter. Physiologic effects of passive hyperventilation on oxygen delivery and consumption. Proc. Soc. Exp. Biol. Med. 140: 1414-1417, 1972.
45. Riley, R. L. Pulmonary function in relation to exercise. In: Science and Medicine of Exercise and Sports, edited by W. R. Johnson. New York: Harper, pp. 162-177, 1960.
46. Rinfret, A. P., and G. F. Doebbler. Physiological and biochemical effects and applications. In: Argon, Helium and the Rare Gases, Vol. II, edited by G. A. Cook. New York: John, Wiley and Sons, p. 727, 1961.
47. Robertson, W. G., and G. L. McRae. Study on man during a 56-day exposure to an oxygen-helium atmosphere at 258 mm. Hg total pressure, VII. Respiratory function, Aerospace Med., 37: 578-582, 1966.
48. Rodgers, S. H., W. O. Fenn, and A. B. Craig, Jr. The oxygen consumption of rat tissues in the presence of nitrogen, helium, or hydrogen. Respiration Physiol. 6: 168-177, 1969.

49. Schatte, C. L., J. P. Jordan, R. W. Phillips, D. P. Clarkson, and J. B. Simmons II. Nonthermal metabolic response of rats to He-O₂, N₂-O₂, and Ar-O₂ at 1 atm. Am. J. Physiol. 225(3): 553-558, 1973.
50. Scholander, P. F. Analyzer for accurate estimation of respiratory gases in one-half cubic centimeter samples. J. Biol. Chem. 167: 235-250, 1947.
51. Schreiner, H. R. General biological effects of the helium-xenon series of elements. Federation Proc. 27: 872-878, 1968.
52. Selkurt, E. E., (Ed.) Physiology. Third ed. Boston: Little, Brown and Co., p. 698, 1971.
53. South, F. E., Jr., and S. F. Cook. Effect of helium on the respiration and glycolysis of mouse liver slices. J. Gen. Physiol. 36: 513-528, 1953.
54. South, F. E., Jr., and S. F. Cook. Argon, xenon, hydrogen and the oxygen consumption and glycolysis of mouse tissue slices. J. Gen. Physiol. 37: 335-341, 1954.
55. Spitler, O. L., S. M. Horvath, K. Kobayashi, and J. A. Wagner. Work performance breathing normoxic nitrogen or helium gas mixtures. Eur. J. Appl. Physiol. 43: 157-166, 1980.
56. Tait, G. T. Exercise performance in men breathing selected mixtures of nitrogen-oxygen and helium-oxygen. State College, Pa: The Pennsylvania State Univ.; 1969. 94 p. Dissertation.
57. Tenney, S. M., and R. E. Reese. The ability to sustain great breathing efforts. Respiration Physiol. 5: 187-201, 1968.
58. Ward, S. A., C. Poon, and B. J. Whipp. Does turbulent airflow constrain compensatory hypernea during metabolic acidosis in exercise? Med. Sci. Sports Exercise. 12(2): 123, 1980.
59. Weast, R. C., (Ed.). Handbook of Chemistry and Physics. 51st ed. Cleveland: Chemical Rubber Publ. Co.; pp. B-15, E-2, 1970.
60. Weiss, H. S., R. A. Wright, and E. P. Hiatt. Embryo development and chick growth in a helium-oxygen atmosphere. Aerospace Med. 36: 201-206, 1965.
61. Wilmore, J. H. Athletic Training and Physical Fitness. Boston: Allyn and Bacon, Inc., p. 251, 1977.
62. Wilson, D., and H. G. Welch. Effects of varying concentrations of N₂/O₂ and He/O₂ on exercise tolerance in man. Med. Sci. Sports Exercise. (In press), 1980.

63. Young, D. R., and S. F. Cook. The effect of helium on the gas exchange of mice as modified by body size and thyroid activity. J. Cellular Comp. Physiol. 42: 319-325, 1953.
64. Zeff, H. J., W. G. Robertson, and B. E. Welch. Study of man during a 56-day exposure to an oxygen-helium atmosphere at 258 mm. Hg total pressure. V. Exercise performance and cardiovascular response. Aerospace Med., 37: 566-571, 1966.

VITA

Alan Glenn Brice was born in Chestertown, Maryland, on March 15, 1953. He attended Chestertown High School and graduated in June 1971. The following September he entered Virginia Polytechnic Institute and State University and June 1975 received a Bachelor of Science degree with a major in Biology.

After working several years as a research assistant at The Johns Hopkins University School of Medicine in Baltimore, Maryland, he entered the Graduate School at The University of Tennessee, Knoxville, to begin study toward a Master of Science degree. He received the Master of Science degree with a major in Zoology in December 1980.