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I am submitting herewith a dissertation written by Alan Glenn Brice entitled "Effect of Respiratory Alkalosis on Hind Limb and in situ Skeletal Muscle Oxygen Uptake in the Dog." I have examined the final copy of this dissertation for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Doctor of Philosophy, with a major in Life Sciences.

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EFFECT OF RESPIRATORY ALKALOSIS ON
HIND LIMB AND IN SITU SKELETAL MUSCLE
OXYGEN UPTAKE IN THE DOG

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ABSTRACT

These experiments were conducted to determine whether changes in skeletal muscle metabolism contribute to the previously reported increase in whole-body O_2 uptake during respiratory alkalosis. Whole-body O_2 uptake was measured by the open-circuit method. The muscle metabolism studies were performed using the hind limb and in situ skeletal muscle preparations in anesthetized and paralyzed dogs. Oxygen uptake of the hind limb and in situ muscle was calculated from measurements of venous blood flow and arterial and venous O_2 concentrations (Van Slyke analysis) during the last 30 min of a 1-hr control period and 1-hr period of respiratory alkalosis. Minute ventilation (hence blood gases and pH) was controlled by a mechanical respirator.

Whole-body O_2 uptake increased significantly ($p < 0.05$) during alkalosis. When control muscle O_2 uptake values were 5.0 ml/kg per min or less, alkalosis resulted in a significant ($p < 0.05$) increase in O_2 uptake over the control values. No increase was observed during alkalosis when the control O_2 uptake was greater than 5.0 ml/kg per min. In all experiments, arterial lactate concentration increased significantly ($p < 0.05$) during alkalosis. Calculation of a positive venous-arterial lactate difference across muscle during the period of alkalosis indicates that skeletal muscle is a source of the elevated blood lactate.

The results of this study indicate that O_2 uptake of resting skeletal muscle is increased during states of respiratory alkalosis. Furthermore, it appears that skeletal muscle contributes to the

elevation in whole-body O_2 uptake that also occurs during alkalosis. The increase in muscle O_2 uptake occurs only when the alkalosis is imposed on muscle in a true basal state.

The mechanism for the increase in O_2 uptake cannot be determined from the present data but some can be suggested. I have proposed that an increase in the rate of substrate cycling may contribute to the increased O_2 consumption observed in this study.

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CHAPTER I

INTRODUCTION

It has long been observed that many higher organisms can survive relatively large fluctuations of the physico-chemical conditions in their external environment, at least for short periods of time. The same, however, cannot be said of the living cells that comprise these organisms. The living cells within higher animals are exposed to and surrounded by an aqueous internal environment, or "fluid matrix" as termed by W. B. Cannon (13). This fluid matrix consists of the blood, lymph, and interstitial fluid. The physico-chemical composition of this extracellular fluid must be maintained within a narrow range to ensure the survival of the organism.

Over one hundred years ago the French physiologist Claude Bernard introduced and stressed the importance of the "fixite du milieu interieur" or the constancy of the body's internal environment. He recognized that the primary goal of the body's organs and organ systems was to maintain this "milieu interieur" relatively constant. Bernard's realization of the importance of maintaining this internal environment constant is apparent from his statement: "It is the fixity of the milieu interieur which is the condition of free and independent life" and that "all the vital mechanisms, however varied they may be, have only one object, that of preserving constant the conditions of life in the internal environment" (6). The American physiologist, W. B. Cannon, later coined the term "homeostasis" in referring to the maintenance of a relatively constant internal environment (13).

Perhaps one of the more important of the regulated variables in

the internal environment is that of pH or hydrogen ion concentration. Hydrogen ion is normally found in extremely low concentrations in the body's fluids, relative to other ions. For example, the hydrogen ion concentration in plasma is about one-millionth that of Na^+ , K^+ , Ca^{++} , Cl^- , HCO_3^- , and phosphate (54). So critical to the survival of an organism is the precise maintenance of hydrogen ion concentration that fluctuations from the normal plasma concentration of 40 nM to greater than 160 nM (pH=6.80) or to less than 10 nM (pH=8.00) usually are not compatible with life (54).

L. J. Henderson stressed the importance of pH in biological systems by stating in 1913 that "acidity and alkalinity surpass all other conditions, even temperature and concentration of reacting substances, in the influence which they exert upon many chemical processes" (31). Henderson's observation, though made over seventy years ago, is most likely an accurate one. The hydrogen ion--or more accurately, hydronium ion, is a highly reactive species. It exerts numerous effects on biological systems, some of which include: alteration in enzyme activity and protein binding, changes in permeability and transport in cell membranes, alterations in the blood clotting mechanism, and in muscle function (43).

Before continuing, I should acknowledge that the classical way of interpreting acid-base physiology is being challenged by a new approach put forth by Peter Stewart (82). I, like almost everyone else, attribute most of the phenomena that occur during acid-base alterations simply to a change in $[\text{H}^+]$. In the new interpretation, changes in $[\text{H}^+]$ (a dependent variable) are explained by the simultaneous changes in the

independent variables (strong ions, PCO_2 , and weak acids). The phenomena that occur during acid-base disturbances may be caused directly by changes in the independent variables rather than by H^+ itself.

In the intact animal, disturbances of acid-base balance in the internal environment may be divided into four general categories. These are respiratory alkalosis, metabolic alkalosis, respiratory acidosis, and metabolic acidosis. This study will focus on respiratory alkalosis and its effects on resting metabolism. This condition arises when alveolar ventilation eliminates carbon dioxide (CO_2) faster than it is produced by the body tissues. This results in a lowering of the arterial CO_2 tension (PaCO_2) and a concomitant decrease in the hydrogen ion concentration to below the normal arterial value of 40 nM. This condition is also known as hypocapnic alkalosis.

There is a considerable amount of data in the literature to support the concept of an increase in whole-body oxygen uptake (VO_2) during respiratory alkalosis. During the past sixteen years there have been numerous reports of a hyperventilation-related increase in whole-body resting VO_2 . This has occurred in anesthetized and non-paralyzed dogs (41, 48, 49, 61, 71, 84), anesthetized and paralyzed dogs (10, 11), anesthetized and panting dogs (78), anesthetized and non-paralyzed monkeys (74), and in awake, normal humans (47). Thus, this response does not appear to be an anesthesia-associated phenomenon nor does it appear to be species specific. Rather, the response is thought to be related to the lowered arterial hydrogen ion concentration that accompanies hyperventilation.

Despite the agreement in the literature of an elevated whole-body $\dot{V}O_2$ during respiratory alkalosis, there has been little or no speculation as to what tissues in the body are responsible for the increased O_2 uptake. Some animals can increase their resting O_2 uptake (heat production) when exposed to cold without any detectable muscle activity. This phenomenon has been termed "non-shivering thermogenesis". Depocas (18) offers evidence that the liver and other abdominal organs are not the site of this extra O_2 consumption. Instead he suggests that skeletal muscle should be considered as the site. This is not to imply that the mechanism of the $\dot{V}O_2$ increase during non-shivering thermogenesis and respiratory alkalosis is the same, rather only that skeletal muscle may be able to alter its resting O_2 uptake in response to external factors.

Skeletal muscle comprises approximately 40-50% of the total body mass in most mammals, including humans and dogs (3, 38). Therefore it would not be unreasonable to expect this tissue to account for a significant portion of the total body resting O_2 uptake. Estimates of the per cent contribution of resting skeletal muscle O_2 uptake to whole-body resting $\dot{V}O_2$ range from 15% to as high as 40% (3, 38, 59). In addition, skeletal muscle is a tissue that is capable of varying its metabolic rate over an extremely wide range. This tissue can increase its O_2 uptake by over fifty times the resting rate (81). Given these general characteristics, skeletal muscle should be considered as a prime source of the purported increased whole-body resting $\dot{V}O_2$ during alkalosis.

Statement of Problem and Hypotheses. There is concordance in the literature that respiratory alkalosis increases whole-body resting $\dot{V}O_2$. However, the identity of the tissue or tissues responsible for this excess O_2 consumption remains unknown. The intent of the experiments to be described was to determine whether changes in skeletal muscle metabolism contribute to the previously reported increase in whole-body O_2 uptake during respiratory alkalosis. The hypotheses are: The resting $\dot{V}O_2$ of skeletal muscle will be increased during respiratory alkalosis as pH increases from approximately 7.40 to 7.60. This elevation contributes to the whole-body increase in O_2 uptake during alkalosis.

CHAPTER II

REVIEW OF THE LITERATURE

The hypothesis that resting skeletal muscle $\dot{V}O_2$ will increase during respiratory alkalosis is based on the premise of an increase in whole-body $\dot{V}O_2$ during this condition. Therefore, the initial part of this review will focus on studies dealing with the effect of respiratory alkalosis on whole-body $\dot{V}O_2$ uptake. Next I will briefly discuss some papers concerned with the effect of metabolic alkalosis on whole-body $\dot{V}O_2$. I will attempt to point out the similarities and differences between the two types of alkalosis and how they relate to the increased $\dot{V}O_2$. The relevant literature is summarized in Table 1. Finally, the limited literature on the hind limb $\dot{V}O_2$ response to respiratory alkalosis and that of in situ skeletal muscle will be discussed.

Respiratory Alkalosis and Whole-Body $\dot{V}O_2$. W. E. Huckabee (41) published data (1958) showing an increase in whole-body $\dot{V}O_2$ in response to a hyperventilation-induced alkalosis. Anesthetized and non-paralyzed dogs had their minute ventilation passively increased by means of a mechanical respirator to three times the control level. Passive hyperventilation resulted in a decrease in mean arterial hydrogen ion concentration ($[H^+]_a$) from the resting value of 40 nM (pH=7.40) to approximately 28 nM (pH=7.56). Oxygen uptake increased by approximately 10% above the control value. Both $[H^+]_a$ and $\dot{V}O_2$ returned to their control levels by the end of a 45-min recovery period.

Interestingly, when the dogs were hyperventilated with gas

Table 1. Effect of alkalosis on whole-body oxygen uptake.

Reference	Year	Species	Type of Alkalosis	[H ⁺] (nM)	PaCO ₂ (Torr)	%Change
Huckabee	1958	Dog	Resp.	28	-	+10
Nahas and Poyart	1967	Dog	Resp.	28	18	+10
			Metabolic	29	34	+10
Mithoefer et al.	1968	Human	Metabolic	28	46	+9.7
Cain	1969	Dog	Resp.	-	-	+6
Karetzky and Cain	1969	Dog	Metabolic	-	-	+
Karetzky and Cain	1969	Dog	Metabolic	-	-	+
Spaich et al.	1969	Dog	Resp.	34	29	+10
Cain	1970	Dog	Resp.	22	17	+19
			Resp.	24	17	+15
			Resp.	24	18	+18
Karetzky and Cain	1970	Human	Resp.	-	*20	+14
Riggs et al.	1972	Monkey	Resp.	28	26	+107
Khambatta and Sullivan	1973	Dog	Resp.	19	14	+25
	1974	Dog	Resp.	18	13	+20
Theye et al.	1977	Dog	Resp.	26	22	+8
Patterson and Sullivan	1978	Dog	Resp.	26	13	+24
			Metabolic	25	34	+21
			Metabolic	25	45	+7

Asterisk indicates value is for alveolar PCO₂.

mixtures containing 5% CO₂, there were no changes in [H⁺]_a or V_{O₂} associated with the three-fold increase in ventilation. It would seem from these data that the increased V_{O₂} is a hydrogen ion and/or PCO₂ related phenomenon. Unfortunately, arterial PCO₂ values, which would have lent further support to this idea, were not reported.

Nahas and Poyart (61) also measured changes in whole-body V_{O₂} in response to alkalosis while investigating the effect of arterial pH change on the metabolic activity of norepinephrine in anesthetized and non-paralyzed dogs. Arterial hydrogen ion concentration was decreased by either administration of sodium bicarbonate or by passive hyperventilation. When sodium bicarbonate was infused (resulting in a metabolic alkalosis), mean arterial [H⁺] fell from the control value of 38 nM (pH=7.42) to 29 nM (pH=7.54) and PaCO₂ remained at about 34 Torr. During the hyperventilation treatment, mean arterial [H⁺] fell to 28 nM (pH=7.55) and PaCO₂ decreased to 18 Torr.

The authors found that a 10% increase in V_{O₂} consistently accompanied the decrease in arterial [H⁺], whether brought about by the bicarbonate infusion or by hyperventilation. Based on these data, it appears that the hyperventilation-associated increase in V_{O₂} is related to the decreased [H⁺]_a rather than the lowered PaCO₂ and bicarbonate concentration that also accompany this condition.

The next major published report of an increased resting V_{O₂} during respiratory alkalosis came in 1969 by S. M. Cain (10). Three anesthetized and paralyzed (succinylcholine) dogs were passively hyperventilated for 30 min. The arterial [H⁺] and PaCO₂ values achieved during this period were not reported. Cain found an average

increase in $\dot{V}O_2$ of approximately 6% during the 30-min test period. These same experiments were then repeated after beta-block (propanolol). The hyperventilation-associated rise in $\dot{V}O_2$ still occurred, suggesting that this increase was not catecholamine related. In another experiment, the increase in $\dot{V}O_2$ was blocked if $PaCO_2$ was kept near 40 Torr by addition of CO_2 to the inspired air during the passive hyperventilation. This finding is similar to that reported previously by Huckabee.

The following year Cain published the results of studies conducted to examine in more detail this phenomenon of a hyperventilation-induced rise in $\dot{V}O_2$ and some possible mechanisms for this increase (11). Three groups of anesthetized and paralyzed (succinylcholine) dogs were used. The first group was progressively hyperventilated by increasing the respirator frequency from 8 to 32 strokes/min in even multiples at 20-min intervals. The procedure was then reversed, again reaching the control rate of 8 strokes/min during the recovery period. During the peak stages of hyperventilation, mean $PaCO_2$ was 17 Torr and arterial $[H^+]$ was 22 nM (pH=7.66). Oxygen uptake increased by 19% from 6.3 ml/kg per min during the control period to 7.5 ml/kg per min during the highest ventilation rate. By the end of the recovery period $PaCO_2$, $[H^+]_a$, and $\dot{V}O_2$ had returned to their control values.

The second group of dogs was hyperventilated at four times the control level for 90 min. Arterial PCO_2 and $[H^+]$ fell to 17 Torr and 24 nM (pH=7.62), respectively. Oxygen uptake increased by 15% after 30 min and remained at this level for the entire 90-min period. After 60 min of recovery, $PaCO_2$, $[H^+]_a$, and $\dot{V}O_2$ returned to their control values.

The third group was hyperventilated at four times the control rate for 30 min. By this time PaCO_2 and $[\text{H}^+]_a$ had fallen to 18 Torr and 24 nM ($\text{pH}=7.61$), respectively, and VO_2 had increased by 18%. Hydrochloric acid was then infused (0.075 mEq/kg per min) for the next 30 min while hyperventilation was continued. This infusion of HCl returned the arterial $[\text{H}^+]$ to its control value and also decreased VO_2 to its control level despite the fact that PaCO_2 remained much below control. Thirty min after this infusion was stopped, both VO_2 and $[\text{H}^+]_a$ returned to about one-half of the initial, pre-infusion hyperventilation values.

This work by Cain is important in several respects. It verified and supported the data of earlier studies that reported an increased whole-body VO_2 during respiratory alkalosis. Secondly, it showed that this elevated VO_2 is not just a transient increase associated with some sort of "unsteady-state" but that it can be maintained for at least 90 min, so long as the respiratory alkalosis persists. Lastly, it attempted to differentiate between the effects of a low PaCO_2 and low $[\text{H}^+]_a$ on the increased VO_2 , suggesting that it is the decreased $[\text{H}^+]_a$ that stimulates O_2 uptake rather than the lowered PaCO_2 .

This increase in O_2 uptake has also been reported in awake human subjects during voluntary hyperventilation. Karetzky and Cain (47) attempted to determine the effect of different minute ventilations, tidal volumes, frequencies, and levels of alveolar PCO_2 on the O_2 cost of breathing. When subjects hyperventilated on room air, alveolar PCO_2 decreased from 38 to 20 Torr while VO_2 increased by 14%. Arterial $[\text{H}^+]$ and PCO_2 were not reported. Based on these data and those obtained when the subjects hyperventilated with CO_2 added to the inspired gas,

the authors concluded that "systemic pH was the predominant determinant of the oxygen uptake at the levels of ventilation studied rather than the mechanical work of breathing."

In 1972 Riggs, Shafer, and Guenter (74) conducted a study to evaluate the physiologic effects of passive hyperventilation on O_2 delivery and consumption. They hypothesized that the increased oxygen-hemoglobin affinity that occurs during alkalotic states may cause tissue hypoxia since the hemoglobin would be unable to release its bound oxygen except at tissue PO_2 levels lower than normal. This decrease in O_2 delivery, if it actually occurs, should be reflected by changes in total body $\dot{V}O_2$. Anesthetized and non-paralyzed rhesus monkeys were passively hyperventilated to approximately three times the control level. Oxygen uptake was calculated by two methods, the open circuit method and by using the Fick principle ($\dot{V}O_2 = \text{cardiac output} \times \text{arterio-venous } O_2 \text{ difference}$). Cardiac output was measured by dye dilution.

Hyperventilation resulted in a decrease in arterial $[H^+]$ from 40 nM (pH=7.39) to 28 nM (pH=7.56) and a fall in $PaCO_2$ from 48 to 26 Torr. Oxygen uptake calculated from both methods increased from 5.5 to 11.4 ml/kg per min. This is a much larger increase than the 10-20% increases reported in previous studies. In fact, the changes in $[H^+]_a$ and $PaCO_2$ are less drastic than those reported by others. It is not known whether this huge difference in $\dot{V}O_2$ is due to physiological differences between the monkeys used in this study and the dogs and humans used in the earlier works, where much smaller increases in $\dot{V}O_2$ were noted.

Fine muscle twitches and fasciculations are sometimes associated with alkalotic states (28). The monkeys used in this study were not paralyzed so the possibility exists that these contractions may have occurred and could account, in part, for the large elevation in O_2 uptake. One must always be aware of the possibility of twitch-induced elevations in resting $\dot{V}O_2$ when non-paralyzed preparations are employed.

Khambatta and Sullivan, in two separate papers, also reported a rise in oxygen uptake during respiratory alkalosis. In the first study (48), anesthetized and non-paralyzed dogs were mechanically ventilated during a 1-hr control period. Arterial $[H^+]$ and PCO_2 values were 37 nM ($pH=7.43$) and 32 Torr, respectively. The animals were then hyperventilated to approximately 3.5 times the control rate for 2 hr. Arterial $[H^+]$ decreased to 19 nM ($pH=7.72$) and $PaCO_2$ to 14 Torr. These changes were accompanied by a 25% increase in whole-body $\dot{V}O_2$. After 2 hr of recovery, $[H^+]_a$, $PaCO_2$, and $\dot{V}O_2$ returned to their control levels. It would have been interesting to follow the time course of the $\dot{V}O_2$ increase during this 2-hr hyperventilation period and then make comparisons with Cain's data (11) where $\dot{V}O_2$ remained elevated by approximately 15% for the entire 90-min hyperventilation period. Unfortunately, $\dot{V}O_2$ was calculated only at the end of the 2-hr period.

The second study (49) dealt with CO_2 production and washout during passive hyperventilation. Specifically, the authors wanted to determine if the change in $\dot{V}CO_2$, once CO_2 washout was complete, was similar to the change in $\dot{V}O_2$ (i.e. what effect does hyperventilation have on the respiratory quotient once washout is complete?). As before, anesthetized and non-paralyzed dogs were passively

hyperventilated to three times the control level for 2 hr. Arterial $[H^+]$ and $PaCO_2$ fell to 18 nM (pH=7.74) and 13 Torr, respectively. Arterial $[H^+]$, $PaCO_2$, $\dot{V}O_2$, and $\dot{V}CO_2$ did not change significantly from the values obtained at the end of Hr 1 to those obtained at the end of Hr 2. Carbon dioxide washout was complete after 70 min. After washout, both $\dot{V}O_2$ and $\dot{V}CO_2$ had increased by approximately the same amount (20%), thus the respiratory quotient was unchanged.

The next report to appear in the literature of a respiratory alkalosis-related increase in whole-body $\dot{V}O_2$ came in 1977. Theye, Gronert, and Heffron (84) examined O_2 uptake of both the whole-body and hind limb during respiratory alkalosis in the dog. For the whole-body experiments, anesthetized and non-paralyzed dogs were hyperventilated for 60 min. Oxygen uptake increased from 5.4 to 5.9 ml/kg per min as arterial $[H^+]$ decreased from 39 nM (pH=7.41) to 26 nM (pH=7.58) and $PaCO_2$ fell from 37 to 22 Torr. This 8% increase is a bit smaller than some of the reports cited earlier but the decreases in $[H^+]_a$ and $PaCO_2$ were not as great either. This paper, however, is most important for the results of the hind limb O_2 uptake response which will be discussed shortly.

In 1978 Patterson and Sullivan (71) published an important work in which they compared the $\dot{V}O_2$ increase stimulated by respiratory alkalosis to that stimulated by metabolic alkalosis (sodium bicarbonate infusion). Anesthetized and non-paralyzed dogs were passively hyperventilated to three times the control ventilation. This treatment was maintained for 1 hr with arterial $[H^+]$ and $PaCO_2$ values reaching 26 nM (pH=7.59) and 13 Torr, respectively. An average increase in

whole-body $\dot{V}O_2$ of 24% was associated with these conditions.

Ventilation was then returned to the control level for another 1-hr period during which arterial $[H^+]$, $PaCO_2$ and $\dot{V}O_2$ returned to their control values.

For the next 2 hr arterial $[H^+]$ was again decreased to the value reached during the hyperventilation period (25 nM), but this time by the continuous infusion of sodium bicarbonate. During this infusion, ventilation was either held at the control level or varied in order to maintain a constant $PaCO_2$. When ventilation was held constant, $PaCO_2$ increased as a result of the bicarbonate dissociation. In either case, bicarbonate infusion resulted in a decrease of arterial $[H^+]$ to 25 nM (pH=7.60).

When ventilation was held constant, with the concomitant rise in $PaCO_2$ to 45 Torr, $\dot{V}O_2$ increased by only 7% despite the decreased $[H^+]$. When ventilated to maintain the control $PaCO_2$ of 34 Torr, $\dot{V}O_2$ increased by 21%, which is quite similar in magnitude to the increases seen during respiratory alkalosis (11, 48, 49). The data from this study support the hypothesis that the extracellular $[H^+]$ is the determinant of O_2 uptake. However, the results from the bicarbonate infusion studies strongly suggest that arterial PCO_2 can greatly influence the O_2 uptake at a given extracellular $[H^+]$, even in the alkaline range.

Metabolic Alkalosis and Whole-Body $\dot{V}O_2$. Before discussing metabolic alkalosis and its effect on O_2 uptake, I would like to first briefly mention some differences between respiratory and metabolic alkalosis. Both types of alkalosis result in a decrease in arterial $[H^+]$ from the normal value of approximately 40 nM. During respiratory

alkalosis, both PaCO_2 and arterial bicarbonate concentration are reduced. In other words, the total CO_2 concentration of the blood is lowered relative to that of normal acid-base conditions. On the other hand, a metabolic alkalosis, such as that which would occur during bicarbonate or sodium lactate infusion, results in elevated PaCO_2 and arterial bicarbonate values (CO_2 concentration in blood is higher than normal).

Two studies that describe the effect of metabolic alkalosis on whole-body VO_2 have already been discussed (61, 71). In these, sodium bicarbonate was infused so as to lower arterial $[\text{H}^+]_a$ to the levels reached during a previous period of respiratory alkalosis. An increase in O_2 uptake during this metabolic alkalosis was reported in both studies. Patterson and Sullivan (71) offered data suggesting that the PaCO_2 can influence the extent of the VO_2 response to a given $[\text{H}^+]_a$ whereas Nahas and Poyart (61) reported no effect of PaCO_2 on VO_2 . A possible reason for this apparent discrepancy may be that in Patterson and Sullivan's study, PaCO_2 reached higher values (46 Torr) than the 34 Torr reported by Nahas and Poyart.

Mithoefer et al. (58) infused sodium bicarbonate into normal, awake human subjects over a 5-min period. During this infusion, minute ventilation, PaCO_2 , and arterial bicarbonate concentration rose as $[\text{H}^+]_a$ fell. Oxygen uptake and VCO_2 increased by 9.7 and 21%, respectively, over the control values during the infusion and 5 min thereafter. This increase in VO_2 during infusion (9.7%) is similar to that reported by Patterson and Sullivan (7%) for the same PaCO_2 (46 Torr) and approximately the same arterial $[\text{H}^+]_a$. All values returned to normal following cessation of infusion.

Karetzky and Cain, in two separate studies (45, 46), reported increases in the $\dot{V}O_2$ of dogs during metabolic alkalosis induced either by sodium bicarbonate, sodium lactate, or sodium pyruvate infusions. Apparently infusions of the sodium salts of lactate or pyruvate exert their alkalinizing effects in the following manner. Lactate and pyruvate leave the extracellular compartment and enter the cell. Bicarbonate ion is then generated on a mole-for-mole basis. It is this bicarbonate ion that can ultimately bind hydrogen ion and reduce its concentration in the extracellular fluid. In all cases the decrease in $[H^+]_a$ was to the same degree following the three types of infusion. All infusions resulted in an elevation of whole-body $\dot{V}O_2$ with lactate and pyruvate infusion yielding the largest change. The authors hypothesized that bicarbonate infusion exerts only an alkalotic stimulatory effect on $\dot{V}O_2$ whereas lactate and pyruvate exert an alkalotic enhancing effect, a stimulation of beta-adrenergic receptors, and an intrinsic metabolic effect because both are readily metabolized substrates (45).

Summary of Whole-Body $\dot{V}O_2$ Response. It is apparent that both respiratory and metabolic alkalosis increase whole-body resting $\dot{V}O_2$. The exact mechanism for this increase is not known at the present time. However, some patterns and clues have emerged from the literature. In all cases of an increased $\dot{V}O_2$, a decrease in arterial $[H^+]$ seems to be the common denominator. It seems that the greater the decrease in $[H^+]$, the greater the increase in $\dot{V}O_2$. This response is not dependent on hypocapnia since $PaCO_2$ remains normal or becomes elevated during

metabolic alkalosis. There is some evidence, however, that the magnitude of the $\dot{V}O_2$ response can be altered by PaCO_2 (71).

Most of the investigators suggest that the increase can be explained by a direct stimulatory effect of a low $[\text{H}^+]$ on key regulatory enzymes such as phosphofructokinase, but they speculate no further than this. The response also appears not to be related to the calorogenic effect of catecholamines because the increase in $\dot{V}O_2$ still occurs during beta-block. Finally, there is little speculation in the literature as to the identity of the tissue or tissues that participate in this response.

Respiratory Alkalosis and Hind Limb $\dot{V}O_2$. There is disagreement in the limited literature regarding the relationship between arterial $[\text{H}^+]$ and hind limb O_2 uptake. One paper reports an increase in hind limb $\dot{V}O_2$ that is proportional to arterial pH (30), while another claimed a decrease in hind limb $\dot{V}O_2$ as arterial pH increases, even though whole-body $\dot{V}O_2$ was elevated (84).

Using an isolated perfused dog hind limb, Harken (30) examined the effect of arterial $[\text{H}^+]$ on $\dot{V}O_2$. The inflow and outflow of blood from the hind limb was limited to the femoral vessels. This was accomplished by placement of a tourniquet around the hind limb but beneath the femoral vessels so as to leave them unoccluded. An extra-corporeal membrane-lung perfusion system was then established in which flow and blood gas tensions could be regulated. Once the perfusion system was functional the dog was killed, thus eliminating all neural and humoral input to the hind limb.

Hydrogen ion concentration was regulated by varying the PCO_2 of

the perfusing blood from 20 to 90 Torr. This resulted in arterial blood $[H^+]$ values ranging from 126 nM (pH=6.9) to 25 nM (pH=7.6). Arterial and venous O_2 concentrations were determined from hemoglobin concentration and saturation measured photometrically with an oximeter. Oxygen uptake was calculated by multiplying flow times the arterio-venous O_2 difference (Fick relationship). This was done after 10-15 min at each arterial CO_2 tension.

A decrease in arterial $[H^+]$ resulted in an increase in hind limb VO_2 . Harken reported a linear relationship between VO_2 and pH in the physiological range. In the range of pH from 7.0 to 7.6, a change of 0.1 pH unit was related to a 10% change in VO_2 . Harken concluded that a decrease in extracellular $[H^+]$ must somehow cause an increase in an ATP-linked cellular process, but he speculated no further.

This first report on hind limb VO_2 during hypocapnic alkalosis follows very closely the patterns mentioned previously for whole-body VO_2 . However, Theye et al. (84) published a paper indicating that an error in Harken's procedure caused the elevated hind limb VO_2 during alkalosis. They used the same preparation as Harken, that is, a sacrificed dog with an isolated hind limb perfused by an extra-corporeal pump-oxygenation system. These investigators found a similar increase in hind limb VO_2 , but they also observed twitches and fasciculations, which undoubtedly contributed to the elevated VO_2 . In this preparation, a mixture of citric acid, sodium citrate, sodium biphosphate, and dextrose (CPD) was added to the perfusate as an anticoagulant. Apparently CPD binds ionized calcium in the plasma because its level decreased nearly ten-fold. A lowered level of

ionized calcium in the extracellular fluids is thought to cause an increase in the excitability of muscle cell membranes with occasional spasms and twitches arising (28). The reduction of extracellular ionized calcium concentration by CPD is enhanced at higher pH values. This could explain, in part, why these investigators observed the muscle contractions during alkalosis. Harken stated that there were no visible muscle contractions during his experiments; however he did not monitor EMG activity, so it is not known for certain whether twitches occurred or not.

When Theye et al. repeated these experiments using heparin instead of CPD as an anticoagulant, the hind limb responded by decreasing its $\dot{V}O_2$ during alkalosis relative to the control $\dot{V}O_2$. In a live preparation with the hind limb intact (self-perfused) and where CPD was not used, Theye et al. found a significant 6% decrease in hind limb $\dot{V}O_2$ even though whole-body $\dot{V}O_2$ increased by 8% during respiratory alkalosis. While this reported 6% decrease in hind limb $\dot{V}O_2$ may be real, it seems questionable in light of the whole-body response. The most obvious question is how can hind limb $\dot{V}O_2$ decrease by 6% at the same time whole-body $\dot{V}O_2$ is increasing by 8%? Since skeletal muscle comprises approximately 40-50% of the total mass of a dog, it is a significant consumer of the resting O_2 uptake (3, 38, 59). If skeletal muscle $\dot{V}O_2$ actually decreases during respiratory alkalosis while whole-body $\dot{V}O_2$ increases, what tissue could possibly be consuming the additional O_2 if it is not skeletal muscle? The authors did not address this paradox in their discussion.

To summarize, the effect of respiratory alkalosis on hind limb $\dot{V}O_2$

is still controversial. It is possible that some of this disagreement may be attributable to the methodology and type of preparation used in these original experiments.

Alkalosis and in situ Skeletal Muscle V_{O_2} . To the best of my knowledge, only one study exists in which the effect of respiratory alkalosis on the V_{O_2} of a resting, isolated muscle preparation was examined. Chapler, Stainsby, and Gladden (15) found no consistent change in the V_{O_2} of the resting gastrocnemius-plantaris muscle group during respiratory alkalosis. However, only three pilot studies were performed and the degree of alkalinization was not as severe as in some of the other studies.

Hirche et al. (33) measured the resting V_{O_2} of the dog gastrocnemius under conditions of metabolic acidosis, $[H^+] = 79 \text{ nM}$, $\text{pH} = 7.1$, and metabolic alkalosis, $[H^+] = 23 \text{ nM}$, $\text{pH} = 7.63$. Oxygen uptake was slightly higher during alkalosis ($3.4 \text{ ml/kg per min}$) relative to that during acidosis ($2.6 \text{ ml/kg per min}$). Unfortunately, no data for normal acid-base conditions were presented.

CHAPTER III

METHODS

This project consisted of five separate series of experiments. These were designed to examine the effect of respiratory alkalosis on: (1) Whole-body O_2 uptake; (2) Hind limb O_2 uptake when no effort was made to restrict the hind limb circulation to a specific set of vessels; (3) Hind limb O_2 uptake when inflow and outflow of blood was restricted to only the femoral artery and vein, respectively; (4) Oxygen uptake of in situ gracilis muscle, and; (5) O_2 uptake of in situ gastrocnemius-plantaris muscle. I will first describe the procedures which were common to all the experiments and then give the detailed protocol for each of the five series.

Anesthesia and Paralysis. Adult mongrel dogs of either sex were used in these experiments. The animals were anesthetized with 30 mg/kg of pentobarbital sodium and maintained at a dosage of 5 mg/kg per hr. The dogs were either intubated with an endotracheal tube whose cuff could be inflated to ensure a tight seal or else they had a tracheal cannula inserted and tied just below the larynx. Esophageal temperature was monitored continuously with a Yellow Springs Tele-Thermometer whose thermistor probe was inserted in the lower third of the esophagus. Esophageal temperature was held relatively constant by use of a heating pad and heat lamp as needed.

As mentioned in the literature review, severe states of alkalosis are sometimes associated with skeletal muscle twitches and/or fasciculations. If these were to occur, measured O_2 uptake would be

higher than the true resting value. Therefore, to prevent the occurrence of these contractions, all dogs were paralyzed with succinylcholine chloride. This was accomplished with an initial intramuscular injection of 20-25 mg and maintained for the duration of the experiment with 0.75 mg/kg per hr (iv). Succinylcholine causes complete paralysis of skeletal muscle by a persistent depolarization of the motor end plate. The doses of succinylcholine used in this study usually have no effect on the cardiovascular system nor on autonomic nerve transmission (8). In addition, succinylcholine is thought not to have an effect on skeletal muscle metabolism (60, 78). After paralysis was induced, the tracheal tube was connected to respirator (Harvard Apparatus, Model 613) and the dog thereafter was ventilated mechanically.

Catheterizations and Blood Sampling Techniques. After induction of anesthesia and intubation, all animals were placed in the supine position where they remained for the duration of the experiment. All dogs in all experiments had a catheter inserted into the right femoral artery high in the femoral triangle. Two three-way stopcocks were attached in series to the end of this catheter. With this arrangement, three ports were made available for blood sampling and/or recording of blood pressure. A Narco Bio-Systems P-1000B pressure transducer was connected to the most distal of these ports. The output from the transducer was sent to a Narco DMP-4B Physiograph and provided a continuous record of arterial blood pressure. The calibration of this transducer was checked periodically during the experiment by means of an internal calibration signal. Before this, the calibration of the transducer was checked against a mercury manometer.

Arterial blood samples for the determination of blood gases, pH, lactate, and catecholamines were obtained in the following manner. To the middle of the three stopcock ports was attached a 10-ml syringe. Before obtaining an arterial sample, the port to this syringe was opened and approximately 10 ml of blood withdrawn through the catheter into this "flush" syringe. Next, the sampling syringe, whose dead space was filled with saline, was attached to the most proximal of the ports. This port was opened and the sample withdrawn. The port was closed and the sample syringe removed. Lastly, the 10 ml of blood in the flush syringe was reinfused into the animal. This process ensured that non-circulating blood in the bore of the catheter was not withdrawn as part of the arterial sample.

In the experiments involving the hind limb and in situ preparations, a catheter was inserted into a jugular vein. This catheter was connected to a funnel held in place by a ringstand. This venous catheter was used for infusion of drugs, saline, and for returning hind limb or in situ muscle venous blood flow to the animal (to be described shortly). For the whole-body $\dot{V}O_2$ experiments, this venous catheter was placed in the right femoral vein. In all but the whole-body $\dot{V}O_2$ experiments, the dogs received heparin as an anti-coagulant at a dosage of 1000-2000 U/kg. This dosage was sufficient to last for the entire experiment.

The placement of the venous catheter used specifically in the determination of hind limb and in situ muscle $\dot{V}O_2$ will be described in the section for that particular type of experiment. The techniques for

measuring blood flow and for venous blood sampling will also be given then.

Blood Gases and pH. Blood samples for the determination of arterial and venous blood gases and pH were drawn anaerobically from the appropriate sampling site into 1-ml plastic tuberculin syringes whose dead space was filled with saline. The sample was immediately measured for PO_2 , PCO_2 , and pH with a Radiometer Blood gas analyzer (BMS3 Mk2) at $37^\circ C$. Hydrogen ion concentration was calculated from the measured pH value. Bicarbonate concentration was calculated from the measured pH and PCO_2 values using the Henderson-Hasselbalch equation.

Oxygen and CO_2 Concentration. Samples for O_2 and CO_2 concentration were drawn anaerobically over a 30-45 sec period from the appropriate site into 3-ml plastic syringes. Approximately 2.5 ml was withdrawn for each sample. The syringe was immediately capped and placed on ice for a later Van Slyke manometric analysis (88). Before analysis, mercury was added to the syringe so that the sample could be thoroughly mixed. Pilot studies indicated that the interval from sampling to Van Slyke analysis (up to 6 hr later) causes no apparent loss of O_2 or CO_2 from the iced sample. Duplicate analyses on refrigerated samples performed up to 12 hr after the initial one resulted in identical values within the limits of variation of the analytical method.

Lactic Acid and Hematocrit. Blood samples for lactate and hematocrit determinations were drawn from the appropriate sites into 1-ml tuberculin syringes. Part of this sample was placed in a spot

plate, the well of which was coated with 50 μ l of a 4% NaF solution. The NaF served to inhibit glycolysis. The blood and NaF were mixed and two separate 50 μ l samples were taken and each placed into 2 ml of cold 7% perchloric acid. These were well mixed by shaking vigorously and were then centrifuged. The supernatant fluid was decanted into clean tubes, then frozen for a later lactate analysis. This analysis was a fluorometric modification of the method of Gutmann and Wahlfeld (27). The samples were analyzed in duplicate. A Turner Model 430 Spectrofluorometer was used in this enzymatic assay.

Hematocrit was determined by the micro-hematocrit method. A Damon IEC Centrifuge and Micro-Capillary Reader were used for this measurement. All reported values are averages of duplicate determinations.

Plasma Epinephrine and Norepinephrine. Two ml of arterial blood to be used for analysis was placed into tubes containing 1.6 mg EGTA and 1.1 mg glutathione. The blood was then gently mixed and then stored on ice until centrifugation. After centrifugation, the plasma was decanted, then frozen until the assay was run. This catecholamine analysis followed a modification of the radioisotope assay by Passon and Peuler (70).

Whole-body $\dot{V}O_2$ Protocol. I am hypothesizing that skeletal muscle contributes significantly to the reported increase in whole-body $\dot{V}O_2$ during respiratory alkalosis. Therefore, it seemed necessary to first demonstrate this increase in my own hands. Four dogs (mean wt. 17.3 kg) were used. Anesthesia, paralysis, and catheterizations have already been described. The trachea was cannulated below the larynx in all four dogs.

$\dot{V}O_2$ and $\dot{V}CO_2$ were determined by the open-circuit method. The expired gases were collected from the exhalation port on the Harvard respirator into Douglas bags. They were analyzed for O_2 and CO_2 fractions with electronic analyzers (Applied Electrochemistry model S-3A and Beckman model LB-2) calibrated with standardized calibration gases previously measured by the Scholander method (77). Pilot studies demonstrated that analyses performed on the electronic analyzers agree to within several one-hundredths of 1% with those of the Scholander method. $\dot{V}O_2$ and $\dot{V}CO_2$ were calculated based on the equations as described by Otis (67):

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

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

where $\dot{V}E$ is expired minute ventilation; FIO_2 , FIN_2 , and $FICO_2$ are fractional inspired concentrations of O_2 , N_2 , and CO_2 , respectively; and FEO_2 , FEN_2 , and $FECO_2$ are fractional expired concentrations of O_2 , N_2 , and CO_2 , respectively. Expired gas volumes were measured by forcing the contents of the bags through a Parkinson-Cowan gas meter. The volumes were then corrected to standard conditions.

The 3-hr experiment consisted of a 1-hr control period (arterial $pH=7.35$; $PaCO_2=32-35$ mm Hg) followed by 1 hr of hyperventilation (arterial $pH=7.60$; $PaCO_2=12-15$ mm Hg) and then another 1-hr control or recovery period. In general, during the control periods, frequency was 9-10 breaths/min and tidal volume was 25 ml/kg body weight. The inspiratory/expiratory ratio of the ventilator was set to 35%. Positive pressure ventilation is known to cause a reduction in arterial

blood pressure (1, 50) especially during hyperventilation. The setting of 35% seemed best at preventing these large decreases. During hyperventilation the frequency was tripled. Arterial blood gases and pH were measured on the Radiometer blood gas analyzer every 10 min and maintained at their desired values by the appropriate adjustment in minute ventilation.

During the first control period, an expired gas sample was collected in a Douglas bag from min 30 to 45 and another sample in a second bag from min 45 to 60. Arterial blood samples for lactate determination were obtained at min 30 and 60. Four expired gas samples were collected during the hyperventilation treatment in four separate bags. These were taken from min 20 to 30, min 30 to 40, min 40 to 50, and from min 50 to 60. Blood samples for lactate analysis were withdrawn at min 20, 40, and 60 of hyperventilation. During the 1-hr recovery period, another expired gas bag was collected from min 45 to 60 and a blood sample at min 45. Hematocrit was checked at random throughout the experiment.

Intact Hind Limb Protocol. In this series of experiments the left hind limb was left intact with no effort made to isolate the hind limb circulation to a specific set of vessels. Although this preparation does not permit an exact quantitative analysis on the effects of alkalosis, it should provide a qualitative one because the hind limb of the dog is approximately 72% skeletal muscle (12). Eight dogs were used (mean wt 20.2 kg). An open-ended catheter was inserted into the left femoral vein high in the femoral triangle. The venous efflux passing through this catheter was returned to the dog via the jugular

vein. A tourniquet was placed around the left ankle to exclude the paw circulation from the preparation.

The venous outflow from the hind limb that passed through this venous catheter was measured by timed collection into a graduated cylinder and also with an electromagnetic flowmeter (Narco, Model RT-500). The cannulating-type flow probe (In Vivo Metric) was located in line on the femoral catheter. Venous samples were drawn through a 20-gauge needle inserted into a rubber adapter located on the venous catheter close to where it exited from the hind limb. Paired arterial and venous blood samples to be analyzed for O_2 and CO_2 concentrations were drawn from the appropriate sites with care taken to accurately match in time the flow measurement with the venous sampling period. Hind limb VO_2 was calculated according to the Fick equation: $VO_2 =$ arterio-venous O_2 difference $\times$ flow.

The experiments consisted of a 1-hr control period and a 1-hr period of hyperventilation. Desired blood gas values were the same as those given previously. Four of the dogs received the 1-hr control period followed by hyperventilation while the others received the treatments in the reverse order. The appropriate samples and measurements for determining hind limb VO_2 were taken at min 30, 40, 50, and 60 of each period. Blood samples for the determination of arterial lactate, epinephrine, and norepinephrine concentrations were obtained at min 0, 30, and 60 of each treatment. Catecholamines were measured in only five of the dogs.

Tied Hind Limb VO_2 Protocol. This series of experiments used five dogs (mean wt. 14.4 kg) and was similar to the intact hind limb series

except for the following differences. Hind limb $\dot{V}O_2$ for each dog was determined at 5 min intervals from min 20 to 60 of each treatment. Arterial lactate concentrations were measured at min 0, 20, 40, and 60 of each period. Plasma catecholamines were not measured. An attempt was made to limit blood flow to and from the hind limb to the femoral artery and vein, respectively. Modifications of this technique to isolate the circulation in both self- and pump-perfused dog hind limbs are commonly employed by other laboratories and have been described previously (12, 30, 84, 91).

This was accomplished in the following manner. A knitting needle threaded with umbilical tape was inserted through the incision site high in the femoral triangle of the left leg to one side of the femur. The needle was withdrawn through a puncture site on the opposite side of the thigh, resulting in the tape being pulled through the entire limb. The same procedure was repeated on the other side of the femur. The tapes were crossed and then tightly tied down with the femur acting as an anchor point so as to occlude all vessels beneath. This tie was made below the femoral vessels and nerve so as to leave them intact. At the end of the experiment the weight of the hind limb tissue perfused was determined by injecting India ink into the femoral artery. The perfused tissue was stained black, then dissected free and weighed. $\dot{V}O_2$ was then expressed as ml O_2 per kg of hind limb tissue.

Isolated Muscle Protocol. The in situ preparations used were those of the gracilis and gastrocnemius-plantaris. For the gracilis preparation, an incision was made parallel and close to the abdominal line and continued down the femoral triangle to beyond the knee. The

gracilis was separated from connective tissue and underlying muscles. The artery and vein distal to the bottom pair of vessels serving the gracilis were tied. The venous outflow was isolated by ligation of all vessels draining into the femoral vein except for the two veins arising from the gracilis itself. Arterial inflow was left intact. The femoral vein was cannulated above the point from where the most proximal of the gracilis veins entered. The venous outflow was returned to the animal via the jugular catheter. The gracilis nerve was left intact.

The gastrocnemius-plantaris muscle preparation has been described previously (79, 80, 81, 90). A medial incision was made through the skin from mid-thigh to the ankle. The muscles which overlie the gastrocnemius-plantaris (the sartorius, gracilis, semitendinosus, and semimembranosus) were doubly ligated and cut between the ties. The venous outflow from the preparation was isolated by ligation of all vessels draining into the popliteal vein except for those from the gastrocnemius itself. Any other outflow vessels from the muscle group not draining into the popliteal vein were also ligated. The arterial inflow was left intact. The popliteal vein was cannulated and the venous outflow returned to the animal via a jugular catheter. The sciatic nerve, which innervates the gastrocnemius, was ligated.

In both of the in situ preparations, all venous flow measurements were by timed blood collections into a graduated cylinder. Muscle temperature was continuously measured by means of a thermistor probe inserted into the muscle group. The experiments consisted of a 1-hr control period followed by 1-hr of hyperventilation. Arterial and

venous blood sample pairs for the determination of muscle $\dot{V}O_2$ were drawn at 5 min intervals from min 20 to 50 of each period. Arterial and venous lactate concentrations were measured at min 20 and 50 of each treatment. Arterial blood gases and pH were measured at min 20, 30, 40, and 50 and venous values at min 25, 35, and 45 of each period. At the end of each experiment the muscle was removed from the animal and weighed.

Statistics. Repeated measures designs were used for the statistical analysis since each animal received each treatment (9). In general, a Treatment (acid-base state) X Treatment (time) X Subjects design was used to test for significant differences between the control and alkalosis periods. A Treatment (acid-base state) X Subjects design was used to test for significant differences between the treatments (Control, Alkalosis, Recovery) in the whole-body $\dot{V}O_2$ experiments. A Duncan's Multiple Range Test was used to determine where differences occurred.

CHAPTER IV

RESULTS

This project consisted of five separate series of experiments. Most of the data are presented in the form of summary tables and figures rather than in the text. The raw data for each of the individual experiments are given in the Appendix.

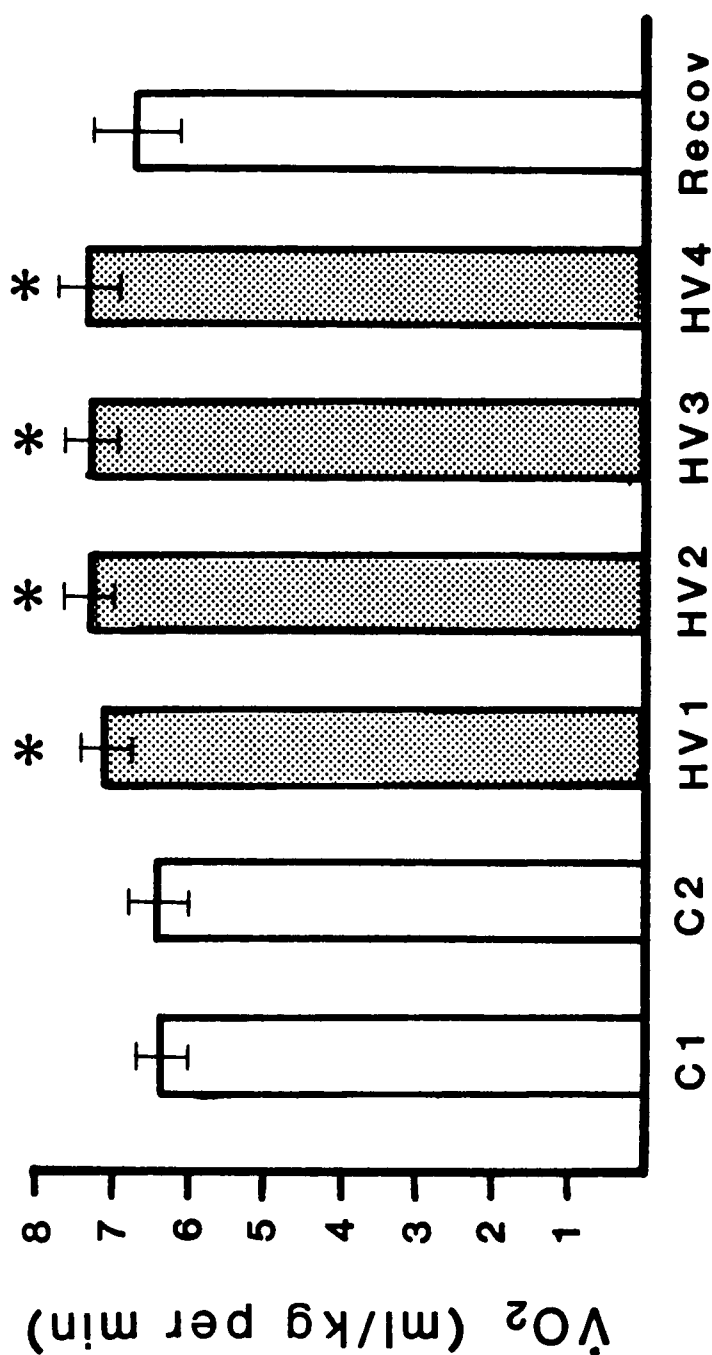
Whole-Body. The results from the whole-body VO_2 experiments are summarized in Table 2. Four dogs (mean wt $17.3 \text{ kg} \pm 1.9 \text{ SE}$) were used in this series. Both VO_2 and lactate concentration increased significantly ($p < 0.05$) during the period of respiratory alkalosis. By the end of the recovery period, the mean values for these two variables had returned to approximately the control level. Figures 1 and 2 give the mean values for VO_2 and lactate, respectively, during each of the measurement periods. Note that within the treatments (Control and Alkalosis), there are no significant differences among sampling periods. Each of the four alkalosis values for VO_2 and the three alkalosis values for lactate are significantly different ($p < 0.05$) from every control and recovery value. The control and recovery values are not significantly different from one another. A comparison of the lactate response to alkalosis among the five different protocols used in this project is given in Figure 3.

Hydrogen ion concentration and PaCO_2 decreased significantly during hyperventilation ($p < 0.05$). The values for $[\text{H}^+]\text{a}$ given in Table 2 of 43, 22, and 40 nM correspond to pH values of 7.37, 7.66, 7.40, respectively. Arterial PO_2 increased significantly during

Table 2. Mean values and SE for selected variables from whole-body VO_2 experiments.

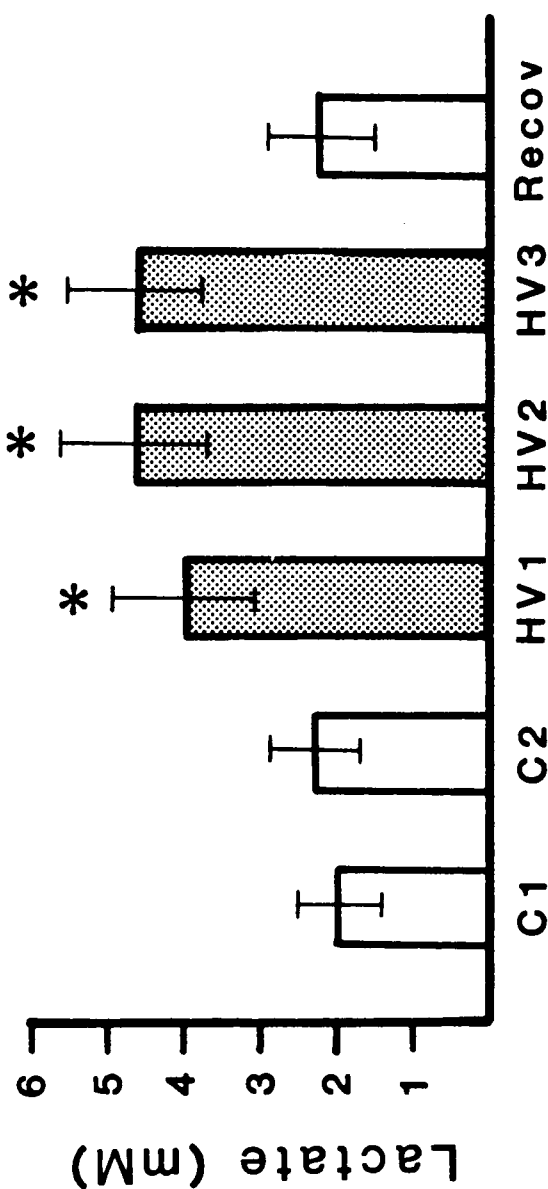
	n	Mean	Std Error of Mean
VO_2 (ml/kg per min)			
Control	8	6.4	0.3
Alkalosis	16	7.3*	0.2
Recovery	4	6.7	0.6
[Lactate] _a (mM)			
Control	8	2.1	0.4
Alkalosis	12	4.4*	0.5
Recovery	4	2.2	0.7
[H ⁺] _a (nM)			
Control	12	43	1
Alkalosis	20	22*	1
Recovery	8	40	2
PaCO_2 (Torr)			
Control	12	36	1
Alkalosis	20	12*	1
Recovery	8	30	3
PaO_2 (Torr)			
Control	12	74	3
Alkalosis	20	88*	2
Recovery	8	66	5
MABP (mm Hg)			
Control	12	141	6
Alkalosis	20	110*	8
Recovery	4	122	14
Esophageal Temp. (°C)			
Control	12	38.4	0.1
Alkalosis	24	38.0	0.1
Recovery	4	39.0	0.4

Asterisk denotes a significant difference from the control and recovery value ($p < 0.05$). n is the total number of observations from all 4 dogs used in this series.



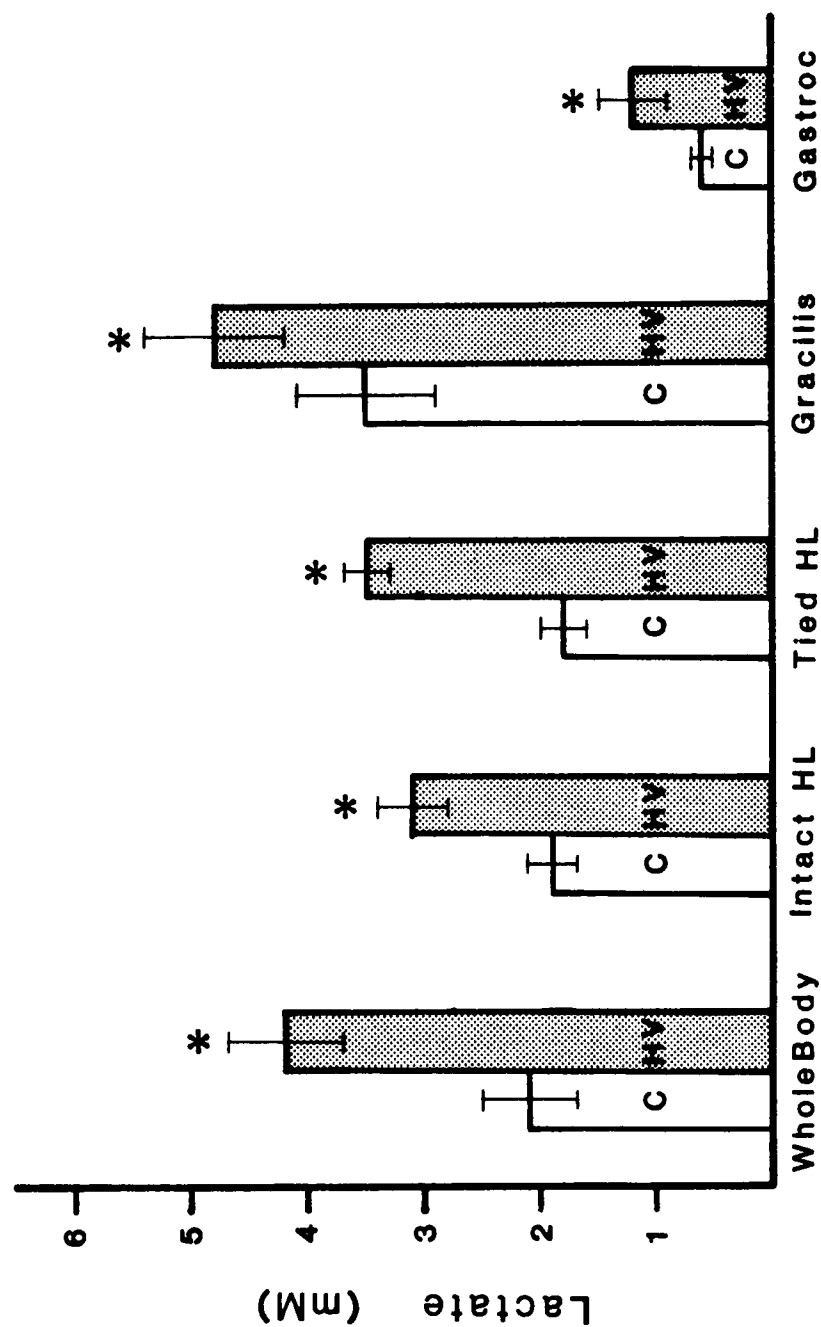
Asterisk denotes a significant difference from control and recovery values ($p < .05$). Values are means ($n=4$) $\pm$ SE. See Methods for actual time of measurement.

Figure 1. Comparison of mean whole-body $\dot{V}O_2$ values during control (C1, C2), hyperventilation (HV1, HV2, HV3, HV4), and recovery.



Asterisk denotes a significant difference from the control and recovery values ($p < .05$). Values are means ($n=4$) $\pm$ SE. See Methods for actual time of measurement.

Figure 2. Comparison of arterial lactate concentration during control (C1, C2), hyperventilation (HV1, HV2, HV3), and recovery.



Asterisk denotes a significant difference from the control value ($p < 0.05$). HL = hind limb

Figure 3. Comparison of arterial lactate response to control (C) and hyper-ventilation (HV) among the different protocols.

hyperventilation ($p < 0.05$). During recovery PaO_2 fell to below the control value, probably as a consequence of the reduction in minute ventilation that was imposed to aid the return of PaCO_2 to near normal. Even at a PaO_2 this low (66 Torr), however, hemoglobin is still approximately 80% saturated with O_2 (75) and should not influence resting metabolic rate. Within the control and alkalosis treatments, PaCO_2 , $[\text{H}^+]_a$, and PaO_2 remained stable, showing little tendency to drift during the 1-hr period, as indicated by the small standard error (Table 2).

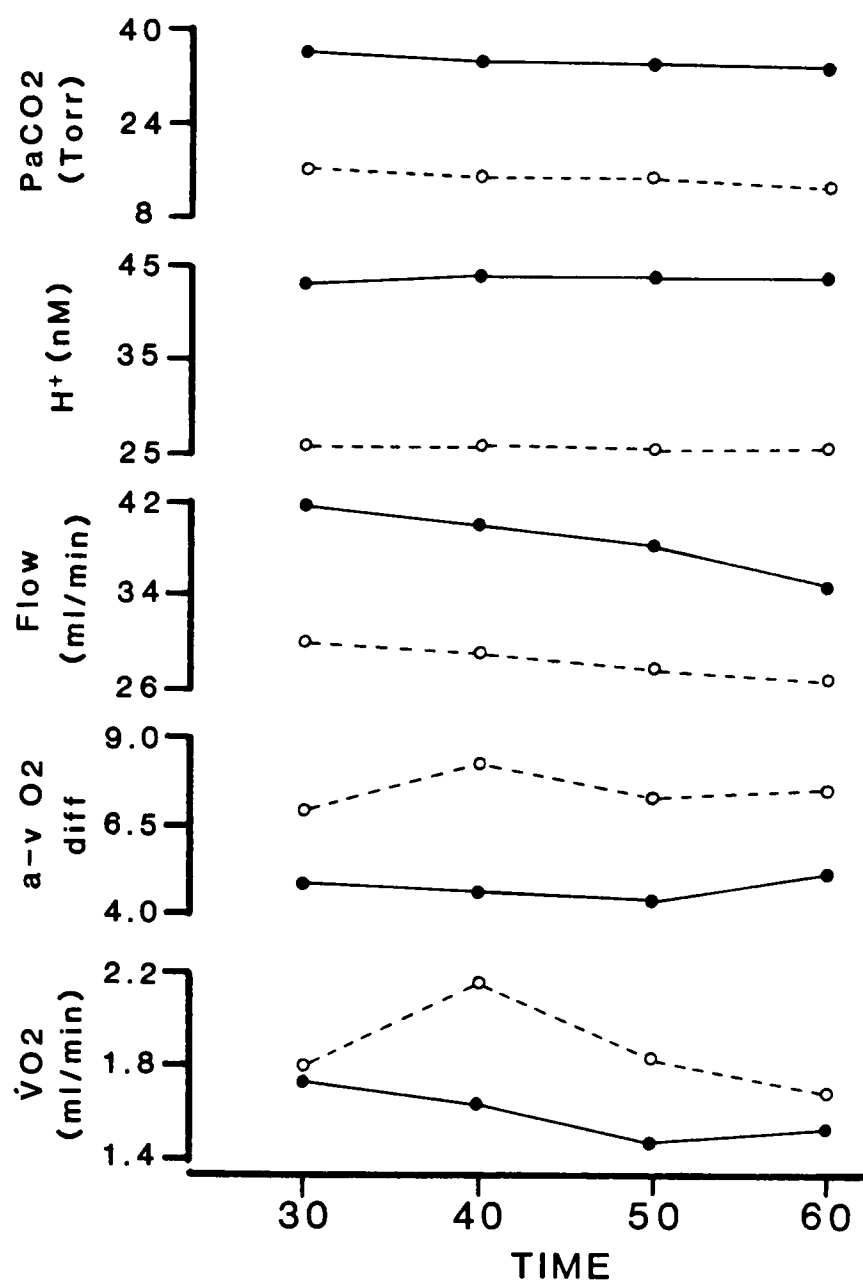
Intact Hind Limb. The data from the intact hind limb experiments are given in Table 3. Seven of the eight dogs used in this series (mean wt $20.2 \text{ kg} \pm 3.0 \text{ SE}$) had a hind limb VO_2 that was higher during hyperventilation. The mean hind limb VO_2 of all eight dogs during alkalosis was 19% greater than that during control ($p < 0.05$). In earlier pilot studies, four out of four dogs responded to the alkalosis with hind limb VO_2 values greater than those measured during the control period. While these experiments are not included as a formal part of the dissertation, they merit mentioning as they provide further evidence of an increased hind limb VO_2 during alkalosis. A summary of these pilot data is given in the Appendix (Table A-5).

Mean lactate concentration was significantly higher ($p < 0.05$) during alkalosis (see Figure 3). This increase occurred in all eight dogs. The mean values for the other variables are listed in Table 3 and will not be given here. The mean values for VO_2 , a-v O_2 difference, flow, $[\text{H}^+]_a$, and PaCO_2 from min 30 to 60 of each treatment are given in Figure 4. The purpose of this diagram is only to describe general trends and

Table 3. Summary of data from intact hind limb experiments.

	n	Control	Alkalosis
VO ₂ (ml/min)	32	1.6 ± 0.2	1.9 ± 0.1*
[Lactate] _a (mM)	16	1.9 ± 0.2	3.2 ± 0.3*
Flow (ml/min)	32	38.7 ± 3.4	28.4 ± 2.4*
CaO ₂ (ml/100 ml)	32	19.2 ± 0.6	20.4 ± 0.5*
CvO ₂ (ml/100 ml)	32	14.5 ± 0.6	12.9 ± 0.7*
a-v O ₂ (ml/100 ml)	32	4.7 ± 0.4	7.5 ± 0.6*
[H ⁺] _a (nM)	32	44 ± 0	26 ± 0*
PaCO ₂ (Torr)	32	34 ± 1	15 ± 0*
[HCO ₃ ⁻] _a (mM)	32	19 ± 0	14 ± 1*
PaO ₂ (Torr)	32	71 ± 2	80 ± 3*
MABP (mm Hg)	32	128 ± 5	110 ± 7*
Esoph Temp (°C)	32	37.3 ± 0.2	36.8 ± 0.3
Hematocrit	16	44 ± 2	43 ± 2

Values are means ± SE. Asterisk denotes a significant difference from the control value ($p < 0.05$). Value for n is the same for both control and alkalosis treatments.



Control values are indicated by a solid line, alkalosis by a dashed line.

Figure 4. Comparison of mean values for $\dot{V}O_2$, a-v O₂ difference, flow, $[H^+]_a$, and PaCO₂ from min 30 to 60 of both control and alkalosis treatments during intact hind limb experiments.

relationships between the two treatments and over time, thus SE have not been included.

Plasma concentrations of epinephrine and norepinephrine were measured in five of the eight dogs used in this series. The mean catecholamine concentrations measured during both control and alkalosis fell within the range of 140-200 pg/ml for epinephrine and 220-400 pg/ml for norepinephrine reported previously by others for resting dogs (51, 72). Thus, plasma levels of epinephrine and norepinephrine did not appear to be affected by the treatment.

Tied Hind Limb. The results from this series of experiments are presented in Table 4. Five dogs were used (mean wt $14.4 \text{ kg} \pm 1.8 \text{ SE}$). There was no overall significant difference ($p=0.56$) in mean hind limb $\dot{V}O_2$ between the treatments. The amount of tissue served by the measured blood flow was determined by staining with India ink so that $\dot{V}O_2$ could be reported per kg of muscle wt. Three out of the five demonstrated a higher $\dot{V}O_2$ during the control period while the other two had higher $\dot{V}O_2$ values during alkalosis (see Table A-2, Appendix).

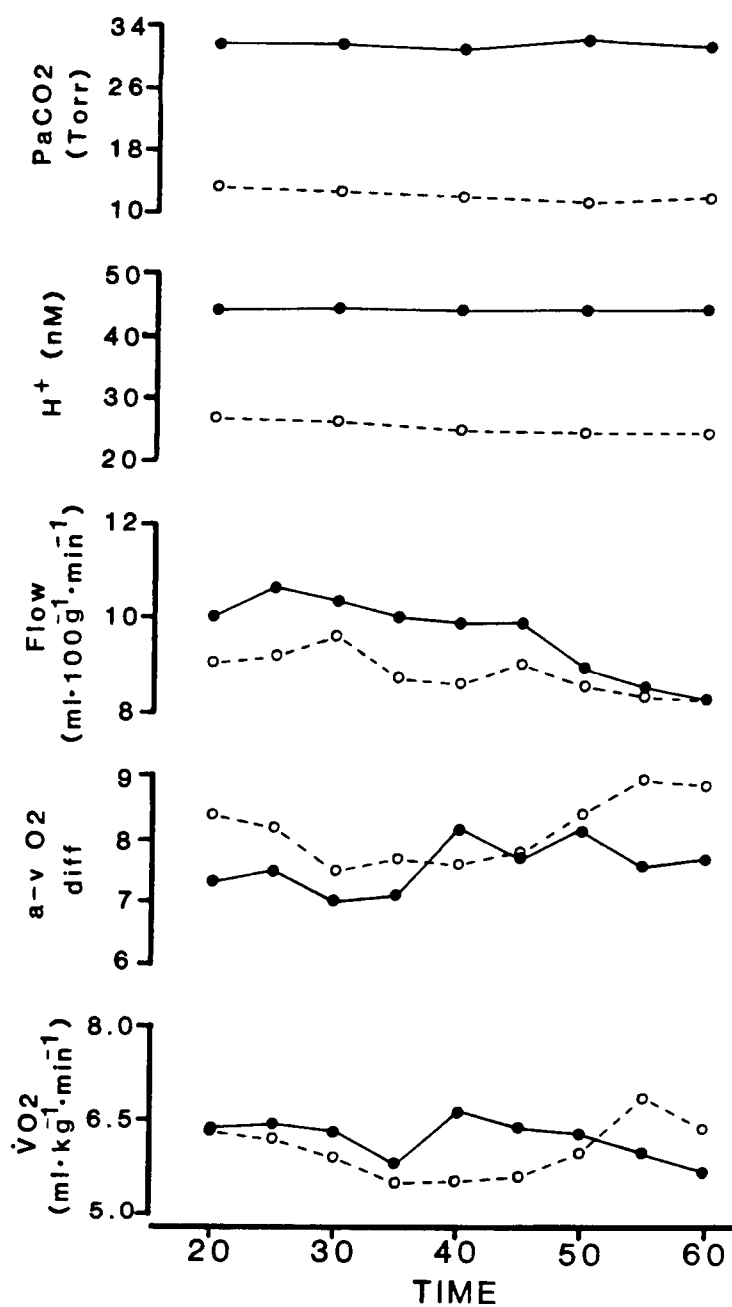
Lactate concentration increased significantly during alkalosis ($p < 0.05$) in all five dogs (see Figure 3). Mean values for the other variables during each treatment are given in Table 4 and will not be mentioned here to avoid repetition. Significant differences are indicated when they occur. Figure 5 gives the mean values for $\dot{V}O_2$, a-v O_2 difference, flow, $[H^+]_a$, and PaCO_2 at 5-min intervals from min 20 to 60 of each treatment.

Gracilis Muscle. A summary of the gracilis data is given in Table 5. Significant differences are indicated when they occur. Three dogs

Table 4. Summary table of data from tied hind limb experiments.

	n	Control	Alkalosis
VO ₂ (ml/min)	45	2.6 ± 0.1	2.6 ± 0.1
VO ₂ (ml/kg per min)	45	6.3 ± 0.3	6.1 ± 0.2
[Lactate]a (mM)	15	1.8 ± 0.2	3.5 ± 0.2*
Flow (ml/min)	45	40.5 ± 2.2	38.0 ± 2.6*
Flow (ml/100 g min)	45	9.7 ± 0.7	8.8 ± 0.6*
CaO ₂ (ml/100 ml)	45	20.1 ± 0.2	21.0 ± 0.3*
CvO ₂ (ml/100 ml)	45	12.5 ± 0.5	12.8 ± 0.6
a-v O ₂ (ml/100 ml)	45	7.6 ± 0.5	8.2 ± 0.5*
[H ⁺]a (nM)	25	45 ± 1	26 ± 1*
[H ⁺]v (nM)	20	48 ± 1	33 ± 2*
PaCO ₂ (Torr)	25	32 ± 0	12 ± 0*
PvCO ₂ (Torr)	20	40 ± 1	21 ± 2*
[HCO ₃ ⁻]a (mM)	25	17 ± 0	11 ± 0*
[HCO ₃ ⁻]v (mM)	20	20 ± 0	15 ± 0*
PaO ₂ (Torr)	25	71 ± 2	94 ± 3*
PvO ₂ (Torr)	20	34 ± 2	30 ± 1*
MABP (mm Hg)	25	111 ± 4	89 ± 3*
Esoph Temp (°C)	25	37.4 ± 0.2	36.0 ± 0.3*
Hematocrit	15	46 ± 1	44 ± 1*

Values are means ± SE. Asterisk denotes a significant difference from the control value ($p < 0.05$). Value for n is the same for both control and alkalosis treatments. Venous values obtained from hind limb outflow.



Control values are indicated by a solid line, alkalosis by a dashed line.

Figure 5. Comparison of mean values for $\dot{V}O_2$, a-v O₂ difference, flow, $[H^+]_a$, and PaCO₂ from min 20 to 60 of both control and alkalosis treatments during tied hind limb experiments.

Table 5. Summary data from gracilis experiments.

	n	Control	Alkalosis
VO ₂ (ml/kg per min)	21	9.1 ± 0.2	7.5 ± 0.3*
WB VO ₂ (ml/kg per min)	6	6.0 ± 0.2	-
[Lactate] _a (mM)	6	3.2 ± 0.6	4.8 ± 0.6*
[Lactate] _v (mM)	6	3.4 ± 0.6	5.5 ± 0.6*
Flow (ml/100 g min)	21	10.7 ± 0.6	6.7 ± 0.4*
CaO ₂ (ml/100 ml)	21	19.9 ± 0.3	21.3 ± 0.4*
CvO ₂ (ml/100 ml)	21	11.0 ± 0.6	9.9 ± 0.3*
a-v O ₂ (ml/100 ml)	21	8.9 ± 0.5	11.4 ± 0.3*
[H ⁺] _a (nM)	12	44 ± 1	28 ± 1*
[H ⁺] _v (nM)	9	50 ± 1	39 ± 1*
PaCO ₂ (Torr)	12	33 ± 1	14 ± 1*
PvCO ₂ (Torr)	9	40 ± 1	27 ± 1*
[HCO ₃ ⁻] _a (mM)	12	18 ± 0	12 ± 1*
[HCO ₃ ⁻] _v (mM)	9	19 ± 0	17 ± 0*
MABP (mm Hg)	9	100 ± 1	79 ± 6*
Muscle Temp (°C)	9	37.8 ± 0.1	37.8 ± 0.1
Esoph Temp (°C)	9	37.6 ± 0.1	36.5 ± 0.1*
Hematocrit	6	44 ± 1	43 ± 1

Values are means ± SE. Asterisk denotes a significant difference from the control value ($p < 0.05$). Value for n is the same for both control and alkalosis treatments. Venous values obtained from gracilis venous outflow. WB VO₂ refers to whole-body O₂ uptake.

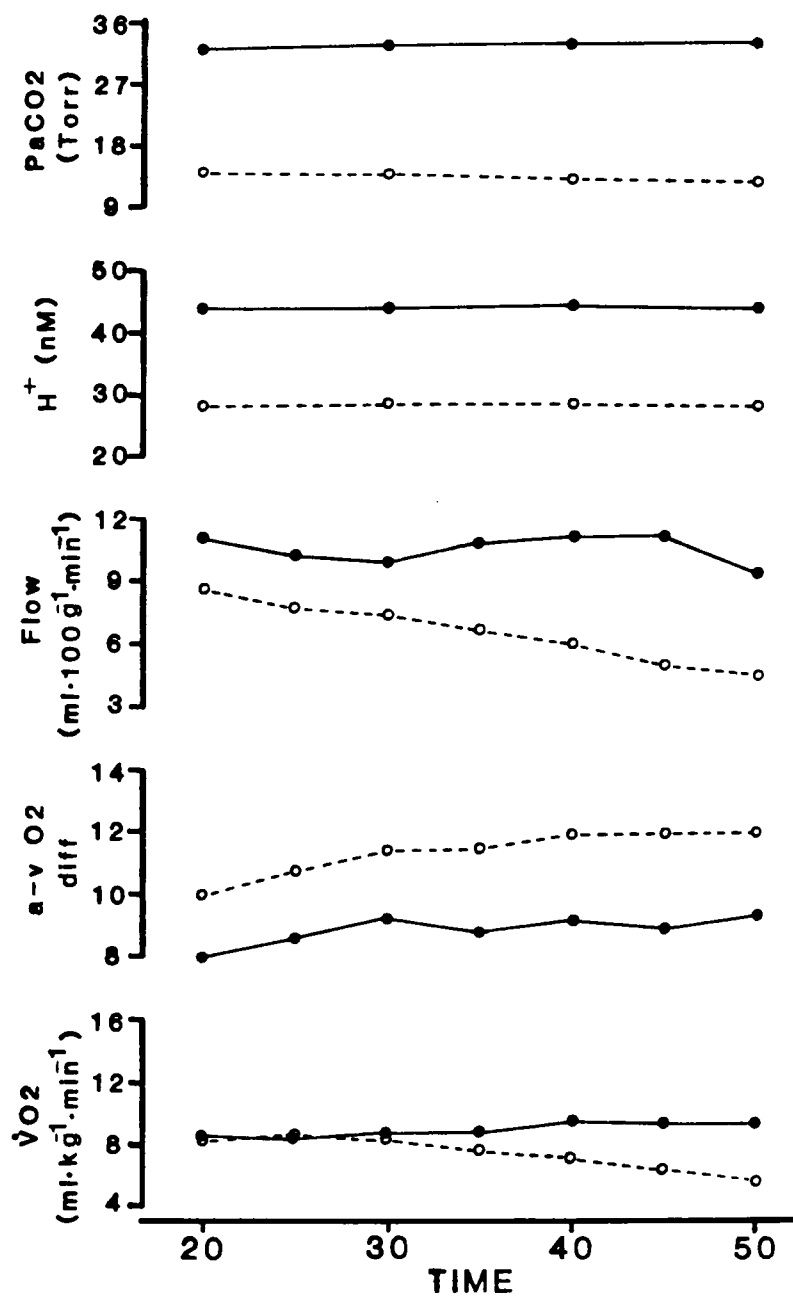
were used in this series of experiments. Mean gracilis weight was 70 g $\pm$ 8.6 SE. Figure 6 gives a comparison of mean values for $\dot{V}O_2$, a-v O_2 difference, flow, $[H^+]_a$, and $PaCO_2$ during min 20 to 50 of the control and alkalosis treatments.

The control gracilis muscle $\dot{V}O_2$ of 9.1 ml/kg per min was significantly higher ($p < 0.05$) than the mean alkalosis value of 7.5 ml/kg per min. In all three experiments, the hyperventilation treatment followed the control period. Figure 7 shows a decline in the high initial $\dot{V}O_2$ values over time (disregarding treatments). This suggests that the significant difference between treatments may be a time effect. This possibility will be discussed more fully in the next section.

Whole-body $\dot{V}O_2$ was measured during the control period of each experiment and is given in the summary table. Lactate concentration increased significantly during alkalosis ($p < 0.05$) although it should be noted that the mean control value was relatively high when compared to the control values measured in the other protocols (see Figure 3).

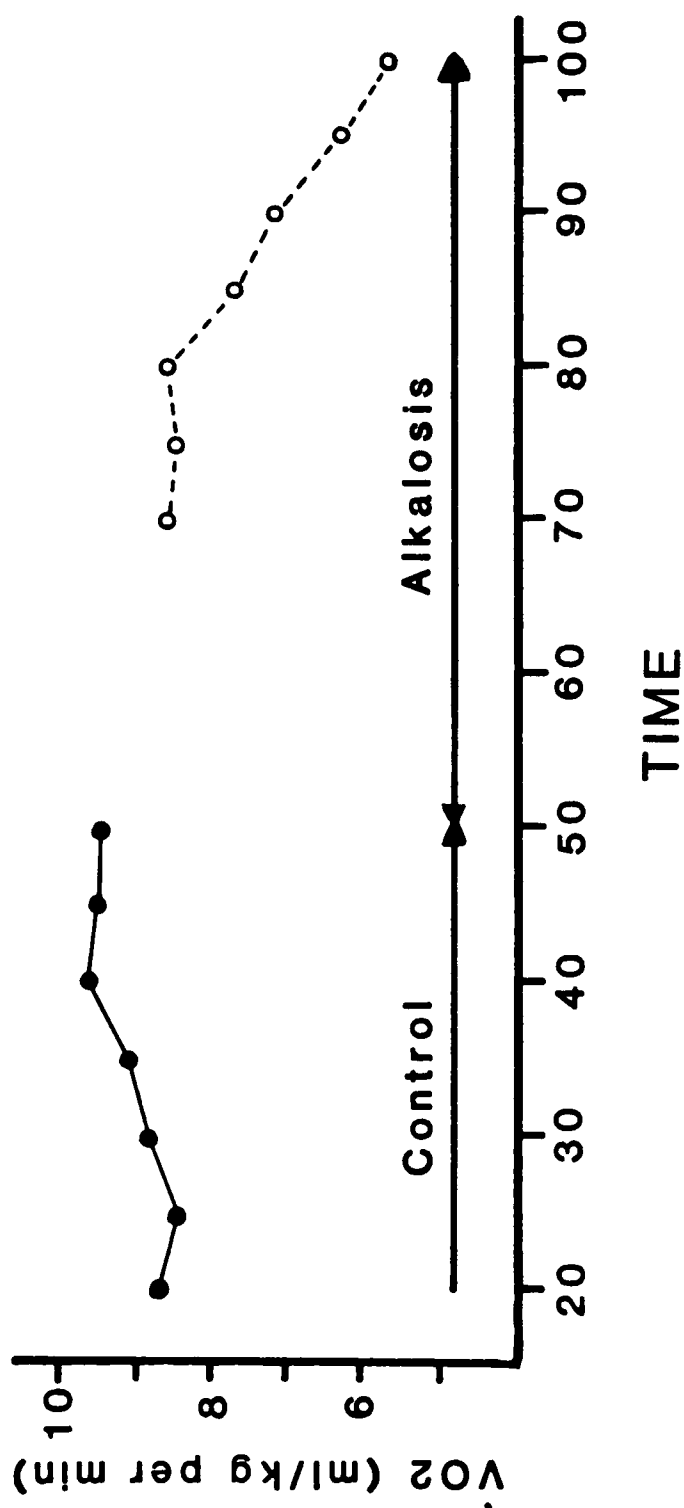
Gastrocnemius-Plantaris Muscle. The results of the gastrocnemius experiments are summarized in Table 6. Significant differences are indicated. Four dogs were used with a mean gastrocnemius weight of 71.6 g $\pm$ 1.4 SE. Figure 8 gives a comparison of mean values for $\dot{V}O_2$, a-v O_2 difference, flow, $[H^+]_a$, and $PaCO_2$ during the last 30 min of both the control and alkalosis treatments.

The mean control gastrocnemius $\dot{V}O_2$ from the first three experiments was 3.6 ml/kg per min and is considered normal for resting muscle. This value increased significantly ($p < 0.05$) during alkalosis to 4.0 ml/kg per min. The mean control $\dot{V}O_2$ from the fourth experiment was



Control values are indicated by a solid line, alkalosis by a dashed line.

Figure 6. Comparison of mean values for $\dot{V}O_2$, a-v O₂ difference, flow, $[H^+]_a$, and PaCO₂ from min 20 to 50 of both control and alkalosis treatments during gracilis experiments.



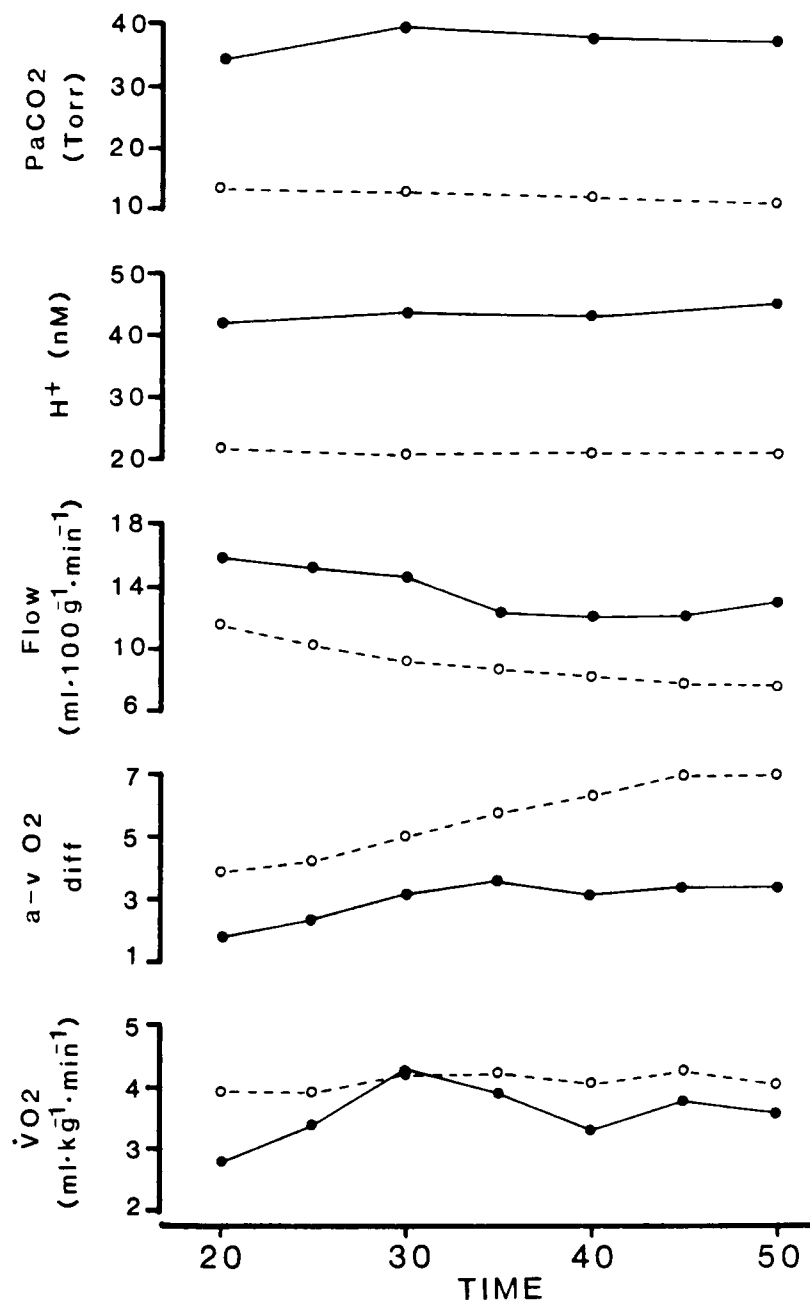
Control values are indicated by a solid line, alkalosis by a dashed line.

Figure 7. Possible time effect on gracilis muscle V02 when initial baseline values are abnormally high.

Table 6. Summary of data from gastrocnemius experiments.

	n	Control	Alkalosis
VO ₂ (ml/kg per min)	21	3.6 ± 0.1	4.0 ± 0.1*
[Lactate]a (mM)	6	0.6 ± 0.1	1.4 ± 0.3*
[Lactate]v (mM)	6	0.6 ± 0.1	1.8 ± 0.3*
Flow (ml/100 g min)	21	13.6 ± 1.0	9.1 ± 0.7*
CaO ₂ (ml/100 ml)	21	20.4 ± 0.4	21.1 ± 0.4*
CvO ₂ (ml/100 ml)	21	17.4 ± 0.4	5.5 ± 0.5*
a-v O ₂ (ml/100 ml)	21	3.0 ± 0.3	5.6 ± 0.8*
[H ⁺]a (nM)	12	43 ± 1	21 ± 1*
[H ⁺]v (nM)	9	44 ± 1	24 ± 1*
PaCO ₂ (Torr)	12	37 ± 1	12 ± 0*
PvCO ₂ (Torr)	9	41 ± 1	16 ± 0*
[HCO ₃ ⁻]a (mM)	12	20 ± 1	14 ± 1*
[HCO ₃ ⁻]v (mM)	9	22 ± 1	18 ± 2*
PaO ₂ (Torr)	12	68 ± 3	69 ± 3
PvO ₂ (Torr)	9	46 ± 1	31 ± 2*
MABP (mm Hg)	9	142 ± 5	111 ± 5*
Muscle Temp (°C)	9	35.4 ± 0.3	35.6 ± 0.1
Esoph Temp (°C)	9	37.8 ± 0.1	37.1 ± 0.2
Hematocrit	6	46 ± 2	44 ± 2

Values are means ± SE. Asterisk denotes a significant difference from the control value ($p < 0.05$). Value for n is the same for both control and alkalosis treatments. Venous values obtained from gastrocnemius venous outflow.



Control values are indicated by a solid line, alkalosis by a dashed line.

Figure 8. Comparison of mean values for $\dot{V}O_2$, a-v O_2 difference, flow, $[H^+]_a$, and $PaCO_2$ from min 20 to 50 of both control and alkalosis treatments during gastrocnemius experiments.

elevated much above normal, thus the data from this experiment were not included in the statistical analysis. I will attempt to justify the exclusion of this experiment from the statistical analysis in the Discussion. The data from this fourth experiment (Dog 4), along with the others, are listed in Table A-4 of the Appendix.

Lactate concentration increased significantly during alkalosis ($p < 0.05$). As shown in Figure 3, the relative per cent increase from control to alkalosis is not unlike the others, but the absolute magnitude of both the control and the alkalosis value is much smaller.

CHAPTER V

DISCUSSION

The results of this investigation support the hypotheses that resting O_2 uptake of skeletal muscle is increased during states of respiratory alkalosis and that skeletal muscle contributes to the increase in whole-body $\dot{V}O_2$ that also occurs. There are, however, several qualifications and limitations to the previous statement that must be addressed. In this chapter I will attempt to: (1) describe and justify the evidence supporting the hypothesis; (2) explain the failure of the tied hind limb and gracilis experiments to support the hypothesis; (3) explain the relationship between the increase in whole-body $\dot{V}O_2$ and that of skeletal muscle; (4) account for the lactate increase during alkalosis, and; (5) describe some possible mechanisms for the increased skeletal muscle $\dot{V}O_2$. Unavoidably, there will be a degree of overlap in the discussion of these points, but I will attempt to address each separately.

Before beginning, I would first like to mention the whole-body $\dot{V}O_2$ studies. The mean control O_2 uptake found in the present experiments, 6.4 ml/kg per min, is in the range of mean values reported by others for anesthetized dogs of similar size, 5.7-6.8 ml/kg per min (10, 11, 12, 49, 61, 71, 84). The mean control whole-body $\dot{V}O_2$ (6.0 ml/kg per min) measured during the gracilis experiments also fits into this range. During respiratory alkalosis, the mean whole-body $\dot{V}O_2$ rose to 7.3 ml/kg per min, an increase of 14%. The per cent increase for each of the four dogs studied in this series was 11, 11, 15, and 18, respectively. These increases fall into the range (8-20%) reported by

others during states of respiratory alkalosis (11, 41, 48, 49, 61, 71, 84).

Data Supporting Hypothesis. When using the intact hind limb preparation, seven of eight dogs demonstrated a hind limb $\dot{V}O_2$ that was higher during respiratory alkalosis. The mean hind limb $\dot{V}O_2$ for all eight dogs during alkalosis (1.9 ml/min) was significantly greater than during control (1.6 ml/min). If the four dogs from the pilot studies are included, eleven out of twelve responded to the alkalosis with a higher limb $\dot{O}_2$ uptake. These results offer strong support for the hypothesis of an elevated resting metabolic rate in skeletal muscle during periods of respiratory alkalosis.

I believe this preparation accurately reflects the metabolic changes induced by respiratory alkalosis. This preparation disturbs the tissue as little as possible and leaves the hind limb circulation and innervation essentially intact. Although a precise quantitative analysis is not possible with this preparation, it does allow a reasonable qualitative assessment of the effects of alkalosis on resting $\dot{O}_2$ uptake.

This preparation does have certain shortcomings. For one, a precise determination of the skeletal muscle mass involved is not possible. The hind limb preparation includes not only skeletal muscle but also bone, fat, and skin, which undoubtedly contribute to the total hind limb $\dot{V}O_2$. However, Cain and Chapler (12) found that skeletal muscle comprises approximately 72% of the entire hind limb mass in mongrel dogs, less the paw which was excluded from the preparation by a tourniquet. Skin and bone account for approximately 13 and 14%,

respectively, of the hind limb mass (12). It is not likely that skin and bone contribute significantly to the total hind limb $\dot{V}O_2$. Honig et al. (38) estimate that only 5% of the measured hind limb blood flow perfuses bone. Pappenheimer found that exclusion of the skin from the circulation of the preparation had no effect on the measured values of hind limb $\dot{V}O_2$ (68). Thus it appears from these data that skeletal muscle is by far the major consumer of O_2 in the dog hind limb, and it is reasonable to attribute the increase in total hind limb $\dot{V}O_2$ to an increase in skeletal muscle O_2 uptake.

The mean control hind limb $\dot{V}O_2$ of 1.6 ml/min falls into the range of values (1.4-2.5 ml/min) reported by others using a similar preparation (68, 69, 76). Using a regression equation formulated by S. M. Cain (personal communication, 1983), it is possible to estimate the muscle weight of the hind limb. This permits muscle $\dot{V}O_2$ to be expressed per kg so that muscle $\dot{V}O_2$ values among the different preparations used in this investigation can be compared. The equation is: $y = 0.0979 + 0.0262x$ where x = dog wt in kg and y = hind limb muscle wt in kg.

When this equation was used to estimate hind limb muscle mass in a group of dogs whose actual values were already known (tied hind limb experiments), the estimated mean value differed from the true value by only 8%. It seems reasonable that hind limb muscle mass in the intact experiments can at least be roughly estimated. When the equation is applied to the eight dogs used in the intact series, the mean hind limb $\dot{V}O_2$, expressed per kg, is 2.5 ml/kg per min. The estimated value during alkalosis is 3.0 ml/kg per min, an increase of 20%.

Another factor inherent to the intact preparation that could be subject to criticism is that the total outflow of blood from the hind limb is not measured because total venous efflux is not limited to just the femoral vein. Oxygen uptake was calculated as the product of flow times the a-v O_2 difference. This means that the values measured in the present experiments should be slightly lower than the true total hind limb VO_2 . I do not think this is a serious problem nor should it have any effect on the outcome or interpretation of the data. The "partial" hind limb VO_2 values measured should be very close to those which would have been obtained had total venous outflow been measured. I offer the following as support.

There are numerous reports in the literature of resting skeletal muscle blood flows ranging from 3.6 to 11 ml/100 g muscle per min (3, 5, 12, 20, 25, 37, 38, 40, 79, 80, 89). Higher flow rates are also reported, but these occur primarily in denervated preparations. Using Cain's regression equation to estimate the hind limb mass of the eight dogs used in this series, mean control flow rate was calculated to be 6.0 ml/100 g muscle per min. This falls into the middle of the range given above and implies that a high percentage of the total venous outflow from the intact hind limb was accounted for in this preparation. In addition, there are many reports of resting skeletal muscle VO_2 values in the 1.1-5.0 ml/kg per min range (5, 15, 19, 20, 25, 32, 33, 34, 35, 37, 38, 40, 79, 83, 90). The control value, based on estimated hind limb mass, in the present experiments was 2.5 ml/kg per min. According to the literature, this would be considered as a normal value for resting muscle. From these flow and VO_2 data, it

seems reasonable to assume that the difference between actual hind limb muscle $\dot{V}O_2$ and that which I measured is not great and should not affect the interpretation of the data.

The last major criticism which could be levied against the intact preparation is the possibility that hyperventilation alters the mass of tissue represented in the measured venous outflow. Based on the data, this possibility does not seem likely. If the increased $\dot{V}O_2$ (in ml/min) measured during alkalosis was only the result of a larger mass of tissue being drained, one would expect the increased $\dot{V}O_2$ to be the result of an increased flow with little or no change in the a-v O_2 difference. Such was not the case. Mean flow decreased by 36% during hyperventilation, probably as a result of a decrease in cardiac output during positive pressure ventilation (1, 50) and also as a result of higher resistance to flow within the hind limb attributable to hypocapnic alkalosis (50). Oxygen uptake can be maintained constant when flow decreases by a compensatory increase of equal magnitude in the a-v O_2 difference. During hyperventilation, a-v O_2 difference increased by 58%, which is 22% more than flow decreased. This indicates a higher metabolic rate per unit mass, not a higher $\dot{V}O_2$ resulting from more tissue being drained.

In summary, the apparent shortcomings with the intact preparation are not as serious as they may have seemed on first inspection. Any inherent sources of error to the preparation are slight and should not have a consequential effect on the outcome. These experiments offer strong evidence in support of the hypothesis of an increased resting skeletal muscle $\dot{V}O_2$ during alkalosis. Perhaps the most convincing support, however, comes from the gastrocnemius experiments.

In the gastrocnemius experiments, measured venous flow consisted only of blood coming from the gastrocnemius-plantaris muscle group. At the end of each experiment, the muscle group was dissected free and its weight determined. During this dissection, no vessels were observed draining into the popliteal vein except for those from the gastrocnemius itself. Thus the entire calculated gross $\dot{V}O_2$ was attributable solely to that particular muscle of known weight. The mean control $\dot{V}O_2$ of 3.6 ml/kg per min agrees well with the range of resting gastrocnemius values, 2.7-4.9 ml/kg per min, reported in the literature (15, 25, 32, 33, 34, 35, 79, 90). There are higher values reported but I do not consider those as true "basal" values. It is for this reason that the data from Dog 4 of this series, whose mean control $\dot{V}O_2$ was 7.4 ml/kg per min, was not included in the statistical analysis. I will have more to say about these high resting values subsequently.

During respiratory alkalosis the mean gastrocnemius muscle $\dot{V}O_2$ uptake increased by 12% to a value of 4.0 ml/kg per min. Thus, in a preparation where the $\dot{V}O_2$ uptake of only skeletal muscle was measured, a distinct difference has been shown. These results, taken together with the data from the intact hind limb experiments, offer convincing support for the hypothesis. The only restriction is that the control or baseline values must be reasonably close to that which would be found in a true physiologic basal condition.

Data Not Supporting Hypothesis. In the tied hind limb experiments, a tourniquet was placed around the upper thigh so that total venous outflow from the limb was forced to exit through the

femoral vein. The data from this preparation indicate no significant difference in O_2 uptake between the treatments (6.3 versus 6.1 ml/kg per min). The conflicting results between this preparation and those of the intact hind limb and gastrocnemius experiments occurred despite the fact that changes in $[H^+]_a$ and $PaCO_2$ when going from control to alkalosis were very similar between the preparations.

It is my feeling that the tied hind limb may not be a suitable preparation to use for studies in which true basal O_2 uptake values are mandatory. Compared to the lower $\dot{V}O_2$ values for resting muscle found in the intact hind limb and gastrocnemius experiments, and those given in the literature (1.1-5.0 ml/kg per min), the tied limb mean control value of 6.3 ml/kg per min seems high. Whether this high value is a reflection of a true basal rate as would occur in an intact animal or as an inherent characteristic of the experimental preparation is open for debate. If all the skeletal muscle in the body had a $\dot{V}O_2$ as high as that measured in the tied hind limb, whole-body $\dot{V}O_2$ would probably be higher than the values reported in the literature (5.7-6.8 ml/kg per min), thus the latter possibility seems most likely.

It is possible that the application of a tourniquet around the upper thigh inflicts trauma on the underlying muscle and nerve tissue. Hofmann (36) suggested that trauma to muscle tissue can significantly increase its O_2 uptake. Using in vitro preparations of human intercostal and various rat skeletal muscles, he demonstrated that intentionally cutting the tissue would nearly double its $\dot{V}O_2$ relative to its metabolic rate in the uninjured state. The tourniquet is also applying pressure on the underlying nerves, including the sciatic

nerve, which contains nearly 90% of the sympathetic innervation to the dog hind limb (16). Cain and Chapler (12) have shown that the hind limb innervation remains functional in spite of the presence of the tourniquet--thus this raises the possibility of occasional impulses arising in the sympathetic fibers contained within the underlying nerves in response to the pressure being applied upon them. Duran and Renkin (20) reported that low frequency stimulation of sympathetic fibers located in the gracilis nerve increased the $\dot{V}O_2$ of resting gracilis muscle by 20-40%. They attributed this increased O_2 uptake to a direct metabolic effect on skeletal muscle by norepinephrine released from the sympathetic fibers.

These are possibilities that certainly must be considered when interpreting the present data. I suggest that the elevated $\dot{V}O_2$ found in the tied hind limb experiments is inherent to the preparation and could mask or overwhelm the treatment effect observed in the other preparations. It may be that the treatment effect can be detected only during true basal conditions and the tied preparation does not permit the measurement of hind limb $\dot{V}O_2$ under such a state. It is for this reason that an overall increase in limb muscle $\dot{V}O_2$ was not observed during alkalosis. It is interesting to note in Table A-2 (Appendix) that the two dogs which showed an increase during alkalosis in this series had the two lowest control $\dot{V}O_2$ values.

The gracilis experiments also failed to show an increase in $\dot{V}O_2$ during alkalosis. In fact, the mean control value of 9.1 ml/kg per min was significantly higher than the alkalosis value of 7.5 ml/kg per min. This mean control value is much greater than the values reported in the

literature for gracilis muscle (1.1-3.1 ml/kg per min) (5, 19, 20, 37, 38, 40, 83). This high value cannot represent the basal $\dot{V}O_2$ of skeletal muscle. In these experiments the control whole-body $\dot{V}O_2$ was 6.0 ml/kg per min. If all the skeletal muscle in the body had a $\dot{V}O_2$ as high as that measured in the gracilis, whole-body $\dot{V}O_2$ would have been much higher than what was measured.

I offer the following as an explanation. The gracilis experiments were the first of the in situ muscle preparations attempted in this project. The high control $\dot{V}O_2$ values most likely are the result of injury and trauma (36) inflicted on the tissue as a result of poor surgical technique that came with lack of experience and unfamiliarity with the preparation. The significantly lower $\dot{V}O_2$ during alkalosis can be explained on the basis of the very high control values. The control period always preceded the alkalosis period. As shown in Figure 7 (page 46), the $\dot{V}O_2$, disregarding treatment, declines over time throughout the experiment. This may indicate the muscle was recovering and stabilizing from the trauma inflicted during the 2-3 hr of surgery.

As in the tied hind limb experiments, an increase in O_2 uptake was not observed during alkalosis, perhaps because this increase may occur only in muscle tissue with a true physiologic basal rate of O_2 uptake. As support, I offer the data in Table 7. In this table the mean $\dot{V}O_2$ value during each treatment from all the hind limb and in situ experiments are given. Note that when the control $\dot{V}O_2$ values are less than 5.0 ml/kg per min, 12 out of 13 dogs show an increased $\dot{V}O_2$ during alkalosis. If the pilot experiments are included, 16 out of 17 show the increase. When the control values are elevated above a reasonable

Table 7. Mean $\dot{V}O_2$ values for individual dogs from all experiments during control and hyperventilation.

Dog	Control	Alkalosis
Intact Hind Limb *		
1	3.3	4.3
2	3.5	3.2
3	1.6	2.4
4	2.3	3.3
5	2.4	2.7
6	1.6	2.0
7	2.8	3.6
8	2.2	2.5
Tied Hind Limb		
1	4.8	5.2
2	7.8	7.2
3	7.6	6.6
4	7.0	6.6
5	4.5	4.6
Gracilis		
1	8.4	7.1
2	9.1	8.1
3	9.9	7.2
Gastrocnemius		
1	3.7	4.4
2	3.8	3.9
3	3.3	3.7
4	7.4	5.7
Pilot Intact Hind Limb *		
1	3.0	3.5
2	2.8	4.8
3	2.9	3.0
4	2.3	2.8

Values are means in ml O_2 /kg per min.

* Muscle mass based on Cain's equation.

control level (5.0 ml/kg per min arbitrarily chosen), 7 out of 7 failed to show the increase during alkalosis. It seems that a basal O_2 uptake is required if skeletal muscle $\dot{V}O_2$ is to be stimulated by an alkalotic condition. If this inference is correct, only the data from the intact hind limb and gastrocnemius experiments are appropriate for testing the hypothesis.

Role of Skeletal Muscle in Increased Whole-Body $\dot{V}O_2$. The approximate per cent contribution to whole-body resting $\dot{V}O_2$ by the various organ and organ systems is thought to be as follows: 1) skeletal muscle, 15-40%; 2) splanchnic organs, 25-40%; 3) brain, 10-20%; 4) kidney, 5-10%; 5) heart, 10-13%, and; 6) skin, 5% (3, 38, 59). Given the small size of the brain, heart, and kidneys in relation to the whole body, it does not seem likely that a change in O_2 uptake of any of these organs would be reflected by a significant change in whole-body $\dot{V}O_2$ at rest. Nor does the literature support the possibility of the liver or other splanchnic organs accounting for the increased whole-body $\dot{V}O_2$ during alkalosis. Studies using in vitro preparations indicate that the O_2 uptake of liver slices is not affected by changes of pH in the range of 6 to 9 (14, 24, 85). Johnson (44) found that total splanchnic $\dot{V}O_2$ was decreased during states of respiratory alkalosis in dogs. The organs involved in this splanchnic measurement included the stomach, intestines, liver, pancreas, and mesentary. He attributed the decrease to a reduction in intestinal $\dot{V}O_2$ because liver $\dot{V}O_2$ was unchanged. Hughes et al. (42) reported a nonsignificant decrease in hepatic O_2 uptake during respiratory alkalosis in anesthetized dogs.

It appears that the liver and other abdominal organs are not responsible for the increased whole-body $\dot{V}O_2$ during alkalosis. Depocas also ruled out these organs as the source of excess O_2 consumption during non-shivering thermogenesis (18). The data from the present study implicate skeletal muscle as being responsible for a large portion of the increase in whole-body $\dot{V}O_2$.

Some rough approximations can be made on the per cent contribution of the increased skeletal muscle $\dot{V}O_2$ to that in the whole-body. These estimates are based on several assumptions for which I do not always have supporting data. I only wish to speculate on the possible extent of muscle's role in the whole-body $\dot{V}O_2$ increase but do not intend to suggest or imply absolute values.

In the intact hind limb experiments, skeletal muscle mean $\dot{V}O_2$ increased by 20%. Whole-body $\dot{V}O_2$, measured in a separate series of experiments, increased 14% during alkalosis. If the assumption is made that skeletal muscle comprises 40-50% of the total body mass, and that all the skeletal muscle in the body is affected to the same extent as that in the hind limb, then the increased skeletal muscle $\dot{V}O_2$ can account for 57-71% of the increase in whole-body $\dot{V}O_2$.

The increase in gastrocnemius mean $\dot{V}O_2$ was not as great as that found in the intact hind limb. There could be several reasons for this. One is that the gastrocnemius responds to alkalosis different from the hind limb, which is composed of other muscles in addition to the gastrocnemius. This is probably not the case since most of the muscles in dogs are thought to consist of mixed fiber types (55). A second possibility is that since the sciatic nerve was ligated,

sympathetic stimulation of muscle O_2 uptake was removed. At any rate, based on a whole-body increase of 14%, 24 to 30% of the whole-body increase could be attributed to skeletal muscle if all the muscle in the body responded to the alkalosis to the same extent as the gastrocnemius.

Lactate Increase During Alkalosis. In 1917 McLeod and Hoover reported that lactic acid appeared in the blood following the injection of an alkalinizing agent such as sodium bicarbonate (57). Anrep and Cannan (1923), in experiments with the Starling heart-lung preparation, and Bock et al. (1932), with humans, found that over- or hyperventilation resulted in a rise in blood lactate (4, 7). Since those early studies, increases in arterial lactate during states of respiratory alkalosis have been widely reported in the literature (11, 21, 23, 26, 30, 41, 92).

Lactate increased significantly during respiratory alkalosis in each series of experiments conducted in this investigation. The per cent increases over the mean control values for the whole-body, intact limb, tied limb, gracilis, and gastrocnemius experiments were 110, 68, 94, 50, and 133%, respectively. These relationships are shown in Figure 3 (p. 36). I have no sound explanation for why the relative increases varied from one series to another. It is interesting to note that the gracilis experiments had the smallest lactate response to alkalosis. This may be related to the high control value relative to the others. The control VO_2 values in the gracilis series were also exceedingly high. The high control lactate and VO_2 values may be related.

Lactate concentration was also determined in the venous outflow from the gracilis and gastrocnemius muscles (see Table 5 and 6, p. 43 and 47). This permitted the venous-arterial lactate difference to be calculated across the muscle. During control conditions, the v-a lactate difference was very small but usually present. It increased by over 3-fold during respiratory alkalosis. This is evidence that skeletal muscle is a source of blood lactate during control and particularly during alkalosis. Andres et al. (3), Cori and Cori (17), Gladden and Yates (25), and Stainsby and Welch (81) have also measured a positive v-a lactate difference across muscle and suggest that resting skeletal muscle should be considered as a major source of arterial lactate . The increase in the v-a lactate difference found in the present study suggests that skeletal muscle is an important source of blood lactate during alkalosis. This is supported by the findings of others (21, 24, 26, 30).

These increased lactate levels are most likely the result of a stimulatory effect of alkalosis on the overall rate of glycolysis. The increased flux through the glycolytic pathway results in an accumulation of pyruvate and NADH faster than they can be used by the mitochondria. The pyruvate could be converted to lactate by a mass action effect. These possibilities are supported by the biochemical properties of the enzymes in the glycolytic pathway.

The activities of the individual glycolytic enzymes have been assessed at different pH values by several groups of investigators. The studies indicate that phosphofructokinase (a key regulatory enzyme in the glycolytic pathway) is the enzyme responsible for the increased

rate of glycolysis during alkalosis (24, 53, 86, 87). This allosteric enzyme catalyzes the rate-limiting conversion of fructose 6-phosphate to fructose biphosphate. The activity of phosphofructokinase (PFK) is significantly increased by a decrease in $[H^+]$, as its pH optimum is in the alkaline range (24, 29, 53, 86, 87). Ui suggested that even a small change of $[H^+]$ in the intracellular fluid plays an important physiological role in the regulation of cellular glycolytic activity (87). Also of interest is the finding that ATP inhibition of PFK is not observed at low values of $[H^+]$ (53, 86, 87). This could help explain how glycolysis can proceed at a high rate when one of its normal allosteric inhibitors, ATP, is expected to be present at a concentration that would normally inhibit PFK.

In addition to the increased production of lactate by muscle cells, it appears that alkalotic states also enhance the rate of lactate efflux from the muscle cell. Mainwood and Worsley-Brown (52) demonstrated that raising the pH of the perfusing fluid of frog sartorius muscle from 6.5 to 8.0 resulted in a 2-3 fold increase in the rate of lactate efflux from the muscle. Hirche et al. (33) reported a 2-fold increase in lactate efflux from contracting dog gastrocnemius muscle during metabolic alkalosis, compared to that during normal acid-base conditions. These studies indicate that lactate efflux from the muscle cell is enhanced when the extracellular fluid is alkalotic. This, along with the stimulation of PFK, may account for the increased levels of blood lactate during respiratory alkalosis.

The phenomenon of increased lactate and pyruvate production during alkalotic conditions is thought to be of homeostatic significance.

This rapid response helps prevent large decreases in $[H^+]$ and has been suggested to form a "third line of defense against excessive changes in blood pH" (4). It is thought that pH control of PFK activity provides a type of feedback control over blood and intracellular $[H^+]$ during alkalotic states (4, 23, 29, 30, 41).

Possible Mechanisms for Increased V_{O_2} . Infusions of sodium lactate and lactic acid have been reported to significantly increase the resting whole-body V_{O_2} of anesthetized dogs (2, 25) and the resting V_{O_2} of dog gastrocnemius muscle (25), respectively. Karetzky and Cain (45) attributed the increase in V_{O_2} during sodium lactate infusion to three factors: alkalosis, beta-adrenergic stimulation, and an intrinsic metabolic effect. Gladden and Yates (25) ruled out the alkalotic effect because they observed an increase in muscle V_{O_2} during lactic acid infusion regardless of whether the animal was alkalotic or acidotic. They suggested that the increase in V_{O_2} may have been caused, in part, by the energy requirement for synthesis of glycogen from lactate in the muscle. This synthesis has been demonstrated previously in the rat hind limb (56).

Blood lactate levels increased during respiratory alkalosis in the present series of experiments. However, before attributing any of the increased V_{O_2} observed in the present experiments to the elevated lactate levels, several factors should be considered. In the infusion studies discussed above, arterial lactate concentrations were greater than 10 mM when the increased V_{O_2} was measured. In the present experiments, lactate concentrations reached during alkalosis were much less than 10 mM, usually in the 3-4 mM range. McLane and Holloszy (56)

found that the rat hind limb removed lactate from the perfusion medium for conversion to glycogen only at concentrations greater than 4 mM.

Secondly, the direction of the muscle-blood lactate concentration gradient probably differed between the present and infusion studies. This may or may not be important. I measured a positive v-a lactate difference across muscle. This indicates release from muscle. During respiratory alkalosis, it is likely that intracellular lactate is elevated as result of increased production via PFK stimulation. The efflux from muscle cells to blood is enhanced by the alkalotic condition (33, 52). When lactate is infused, the lactate concentration of the blood is probably higher than in the resting muscle cells. In this condition lactate moves from the blood to the tissues. This is supported by the finding of a negative v-a lactate difference across resting muscle during infusion (25).

Because the conditions are so different between the present and the infusion studies, it is difficult to say whether the mechanism that increases $\dot{V}O_2$ during lactate infusion could also be operational when lactate is elevated via respiratory alkalosis. Further work is required before the respiratory alkalosis-induced increase in lactate can be credited for the increased $\dot{V}O_2$ observed in the present studies.

Elevated levels of plasma catecholamines or increased sympathetic stimulation of skeletal muscle could cause increased O_2 uptake in muscle. I do not consider these as likely explanations for the increases observed in the intact hind limb and gastrocnemius experiments. Epinephrine and norepinephrine concentrations were measured in the intact hind limb experiments. The concentration of

these catecholamines was not affected by respiratory alkalosis. Unfortunately they were not measured in any other experiments. However, several lines of evidence support the idea that catecholamines are not altered by alkalosis. Nahas et al. (60) found that plasma catecholamine levels remained constant in anesthetized dogs during alkalosis. Later, Nahas and Poyart (61) reported that the metabolic effect of infused norepineprine was not potentiated by alkalosis. Fiorca et al. (22), when studying the effect of heat stress on sympathetic nervous activity in dogs, concluded that hypocapnic alkalosis is associated with suppression of the sympatho-adrenomedullary system. Based on the data from the present study and that found in the literature, it appears that circulating catecholamines are not increased by alkalotic conditions nor are their effects potentiated.

Other evidence indicating that catecholamines are not involved comes from Cain (10). When he hyperventilated anesthetized dogs, a rise in whole-body $\dot{V}O_2$ occurred. When the same experiments were repeated after beta-block (propanolol), the increase in $\dot{V}O_2$ still occurred, suggesting that catecholamines were not involved in the whole-body $\dot{V}O_2$ increase. An increase in sympathetic tone to the muscle cannot explain the entire difference either. In the gastrocnemius experiments the sciatic nerve was ligated, yet an increase in $\dot{V}O_2$ still occurred during alkalosis.

A stimulation of resting $\dot{V}O_2$ by alkalosis requires an increased flux through the entire oxidative pathway. This should result in an increased production of adenosine triphosphate (ATP). However, an

increase in the intracellular ATP concentration should exert negative feedback on the various regulatory enzymes in the pathway. In glycolysis alone, hexokinase, PFK, and pyruvate kinase are inhibited by ATP (29, 53, 73, 86, 87). How then, can the elevated metabolic rate continue during alkalosis? One possibility could be an uncoupling of oxidative phosphorylation in the electron transport chain. This phenomenon occurs when electron transport continues as usual but ADP is not phosphorylated to ATP. I do not think this is the explanation because ATP accumulation could still occur via substrate level phosphorylation in glycolysis. Instead, I propose an increase in ATP hydrolysis in response to an increase in the rate of substrate or "futile" cycling.

Evidence for the existence of substrate cycles was first obtained in 1966 by Newsholme and Underwood (66). Since that time, more and more evidence has been obtained to show that substrate cycles occur in several types of cells (kidney, liver, adipose, skeletal muscle). A substrate cycle occurs when a non-equilibrium reaction in the forward direction of a pathway is opposed by a second non-equilibrium reaction in the reverse direction of the pathway (63). The reactions occur simultaneously, and each is catalyzed by a separate enzyme. The net result of such a cycle is the dephosphorylation of ATP. Newsholme refers to this net hydrolysis of ATP as an "energy leak" (63). These cycles and their role in metabolic control have been described in several reviews (62, 64).

Newsholme proposes that the primary function of substrate cycles is to increase the sensitivity of non-equilibrium reactions to changes

in the concentration of metabolic regulators (62). Sensitivity refers to the magnitude of change in the rate of a reaction in response to a change in the concentration of an allosteric modulator, either positive or negative. An increased sensitivity, via a substrate cycle, permits the flux through a metabolic pathway to increase enormously without requiring a large change in the concentration of a metabolic regulator. Other suggested roles for substrate cycles include heat generation for maintaining body temperature when exposed to cold environments and a biochemical mechanism for maintaining a relatively constant body weight (62, 64).

There are a large number of possible substrate cycles that could occur if all metabolic pathways are considered. Newsholme and Crabtree (64) consider the cycles occurring between the following as perhaps the most important: fructose 6-phosphate and fructose bisphosphate in liver, kidney, and muscle; glucose and glucose 6-phosphate in the liver; triglyceride and fatty acid in white adipose tissue; triglyceride and fatty acid in muscle; triglyceride and fatty acid between adipose tissue and liver; and, hexose monophosphate and glucose in liver and muscle. Certain enzymes also cycle between active and inactive forms--phosphorylase a and b for example.

The fructose 6-phosphate/fructose bisphosphate cycle is considered as one of the most active and costly for ATP hydrolysis. It is known to occur in mammalian skeletal muscle. In this cycle, PFK catalyzes the phosphorylation of fructose 6-phosphate to fructose bisphosphate. In the opposing reaction, fructose bisphosphatase catalyzes the dephosphorylation of fructose bisphosphate back to fructose

6-phosphate. In the resting, unstressed, intact animal the rate of this cycle is thought to be very low. The rate of this cycle in skeletal muscle can be increased by several factors. Newsholme has suggested that circulating hormones such as epinephrine, glucagon, and glucocorticoids, in addition to substances released locally in the muscle such as norepinephrine and prostaglandins can control the rate of cycling (62).

In addition to the nervous and hormonal mechanisms for increasing cycling, Newsholme and Jones (65) suggested that the fructose 6-phosphate/fructose biphosphate cycle may be increased by a pH or $[H^+]$ effect. Given the characteristics of the two enzymes involved in this cycle, this mechanism for increasing cycling rate is plausible. I have already discussed in detail the stimulatory effect of a lowered $[H^+]$ on the activity of PFK. The second enzyme in this cycle, fructose biphosphatase, has been shown to have a pH optimum in the alkaline range (39). Thus, a decrease in $[H^+]$, as would occur during respiratory alkalosis, could simultaneously increase the activities of both enzymes involved in this cycle. This would result in an increased rate of cycling and a concomitant increase in ATP hydrolysis.

If a decreased $[H^+]$ can indeed increase the rate of the fructose 6-phosphate/fructose biphosphate cycle, the next question becomes: Can this increased rate of ATP hydrolysis account for the increase in $\dot{V}O_2$ observed during alkalosis? Based on the data in the literature, the answer is yes. Newsholme and Crabtree (64) and Newsholme (62) have shown that the amount of heat produced or extra O_2 consumed during maximal rates of cycling can be large. Maximum cycling of just the

fructose 6-phosphate/fructose biphosphate cycle can require as much O_2 as the basal metabolic rate itself--that is, it could cause a doubling of the resting VO_2 (62).

A short series of calculations will demonstrate this. The maximum activity of fructose biphosphatase is 2.0 $\mu\text{mol}/\text{min}$ per g muscle. That of PFK is 60 $\mu\text{mol}/\text{min}$ per g muscle. These determinations were made on human quadriceps muscle (62). I will assume that the activity of fructose biphosphatase is similar in dog muscle. The highest possible rate of cycling between fructose 6-phosphate and fructose biphosphate is 2.0 $\mu\text{mol}/\text{min}$ per g muscle. One turn of the cycle causes the hydrolysis of 1 molecule of ATP. This means the rate of hydrolysis is 2.0 $\mu\text{mol ATP}/\text{min}$ per g muscle (63). Expressed per kg, it becomes 0.002 moles ATP/min per kg muscle. Put another way, 0.002 moles ADP/min per kg muscle need to be phosphorylated to resupply ATP.

If the P/O ratio is assumed to be 3, then 6 moles of ADP will be phosphorylated to ATP for each mole of O_2 consumed. Based on the previous calculation, the phosphorylation of 0.002 moles of ADP/min per kg muscle should require 0.00033 moles O_2/min per kg muscle. Expressed another way, if the cycle was maximally active, O_2 uptake could be elevated by approximately 7.5 ml O_2/kg per min. It is doubtful that this cycle operates at its maximal rate in vivo; a value of 20-30% is likely to be the highest rate attained (62). If the cycle operates at 20-30% of maximum, O_2 uptake would be increased by 1.5-2.5 ml/kg per min. The operation of this cycle alone could account for the increase in VO_2 found in this investigation.

Conclusion and Summary. The results of this study indicate that O_2 uptake of resting skeletal muscle is increased during states of respiratory alkalosis. Furthermore, it appears that skeletal muscle contributes significantly to the elevation in whole-body $\dot{V}O_2$ that also occurs during alkalosis. This increase in muscle $\dot{V}O_2$ occurs only when the alkalosis is imposed on muscle in a true basal metabolic state. If the resting $\dot{V}O_2$ is elevated, for any reason, an increase in O_2 uptake during alkalosis is not likely to occur.

The mechanism for the increase in O_2 uptake cannot be determined from the present data but some can be suggested. I have proposed that an increase in the rate of substrate cycling may contribute significantly to the increased $\dot{V}O_2$ observed in this study. Verification will require direct demonstration using isotopic techniques.

LIST OF REFERENCES

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1. Abel, F. L., and J. A. Waldhausen. Effects of anesthesia and artificial ventilation on caval flow and cardiac output. J. Appl. Physiol. 25:479-484, 1968.
2. Alpert, N. R., and W. S. Root. Relationship between excess respiratory metabolism and utilization of intravenously infused sodium racemic lactate and sodium L (-) lactate. Am. J. Physiol. 177:455-462, 1954.
3. Andres, R., G. Cader, and K. L. Zierler. The quantitatively minor role of carbohydrate in oxidative metabolism by skeletal muscle in intact man in the basal state. Measurements of oxygen and glucose uptake and carbon dioxide and lactate production in the forearm. J. Clin. Invest. 35:671-682, 1956.
4. Anrep, G. V., and R. K. Cannan. The concentration of lactic acid in the blood in experimental alkalaemia and acidaemia. J. Physiol. 58:244-258, 1923.
5. Beer, G., and L. R. Younce. Blood flow, oxygen uptake, and capillary filtration in resting skeletal muscle. Am. J. Physiol. 223:492-498, 1972.
6. Bernard, C. Lecons sur les phenomenes de la vie commune aux animaux et aux vegetaux. Paris: Bailliere, 1878, p. 114-121.
7. Bock, A. V., D. B. Dill, and H. T. Edwards. Lactic acid in the blood of resting man. J. Clin. Invest. 11:775, 1932.
8. Booth, N. H., and L. E. McDonald (Eds). Veterinary Pharmacology and Therapeutics. Fifth ed. Ames: The Iowa State University Press, 1982, p. 133-142.
9. Bruning, J. L., and B. L. Kintz. Computational Handbook of Statistics. Glenview: Scot, Foresman, 1968.
10. Cain, S. M. Diminution of lactate rise during hypoxia by PCO_2 and B-adrenergic blockade. Am. J. Physiol. 217:110-116, 1969.
11. Cain, S. M. Increased oxygen uptake with passive hyper-ventilation of dogs. J. Appl. Physiol. 28:4-7, 1970.
12. Cain, S. M., and C. K. Chapler. O_2 extraction by hind limb versus whole dog during anemic hypoxia. J. Appl. Physiol.: Respirat. Environ. Exercise Physiol. 45:966-970, 1978.
13. Cannon, W. B. The Wisdom of the Body. New York: W. W. Norton and Co., Inc., 1939, p. 24-40.

14. Canzanelli, A., M. Greenblatt, G. A. Rogers, and D. Rapport. The effect of pH changes on the in vitro O_2 consumption of tissues. Am. J. Physiol. 127:290-295, 1939.
15. Chapler, C. K., W. N. Stainsby, and L. B. Gladden. Effect of changes in blood flow, norepinephrine, and pH on oxygen uptake by resting skeletal muscle. Can. J. Physiol. Pharmacol. 58:93-96, 1980.
16. Clonninger, G. L., and H. D. Green. Pathways taken by the sympathetic vasomotor nerves from the sympathetic chain to the vasculature of the hind leg muscles of the dog. Am. J. Physiol. 181:258-262, 1955.
17. Cori, C. F., and G. T. Cori. Mechanisms of epinephrine action. IV. The influence of epinephrine on lactic acid production and blood sugar utilization. J. Biol. Chem. 84:683-698, 1929.
18. Depocas, F. Calorigenesis from various organ systems in the whole animal. Fed. Proc. 19, Suppl. No. 5:19-24, 1960.
19. Duran, W. N., and E. M. Renkin. Oxygen consumption and blood flow in resting mammalian skeletal muscle. Am. J. Physiol. 226:173-177, 1974.
20. Duran, W. N., and E. M. Renkin. Influence of sympathetic nerves on oxygen uptake of resting mammalian skeletal muscle. Am. J. Physiol. 231:529-537, 1976.
21. Eldridge, F., and J. Salzer. Effect of respiratory alkalosis on blood lactate and pyruvate in humans. J. Appl. Physiol. 22:461-468, 1967.
22. Fiorca, V., P. F. Iampietro, E. A. Higgins, and R. Moses. Sympathico-adrenomedullary (SAM) activity in dogs during acute heat exposure. J. Appl. Physiol. 22:16-20, 1967.
23. Garcia, A. C., Y. L. Lai, B. A. Attebery, and E. B. Brown, Jr. Lactate and pyruvate accumulation during hypocapnia. Resp. Physiol. 12:371-380, 1971.
24. Gevers, W., and E. Dowdle. The effect of pH on glycolysis in vitro. Clin. Sci. 25:343-349, 1963.
25. Gladden, L. B., and J. W. Yates. Lactic acid infusion in dogs: effects of varying infusate pH. J. Appl. Physiol.: Respirat. Environ. Exercise Physiol. 54:1254-1260, 1983.
26. Goldstein, P. J., D. H. Simmons, and D. P. Tashkin. Effect of acid-base alterations on hepatic lactate utilization. J. Physiol. 223:261-278, 1972.

27. Gutmann, I., and A. W. Wahlefeld. L-(+)-lactate determination in lactate dehydrogenase and NAD. In: Methods of Enzymatic Analysis (2nd ed.), edited by H. U. Bergmeyer. New York: Academic, 1974, p. 1464-1468.
28. Guyton, A. C. Textbook of Medical Physiology. Fifth edition. Philadelphia: W. B. Saunders Co., 1976, p. 127.
29. Halperin, M. L., H. P. Connors, A. S. Relman, and M. L. Karnovsky. Factors that control the effect of pH on glycolysis in leukocytes. J. Biol. Chem. 244:384-390, 1969.
30. Harken, A. H. Hydrogen ion concentration and oxygen uptake in an isolated canine hindlimb. J. Appl. Physiol. 40:1-5, 1976.
31. Henderson, L. J. The Fitness of the Environment. Boston: Beacon, 1913, p. 153.
32. Hirche, Hj., D. Grun, and W. Waller. Utilization of carbohydrates and free fatty acids by the gastrocnemius of the dog during long lasting rhythmical exercise. Pflugers Arch. 321:121-132, 1970.
33. Hirche, Hj., V. Hombach, H. D. Langohr, U. Wacker, and J. Busse. Lactic acid permeation rate in working gastrocnemii of dogs during metabolic alkalosis and acidosis. Pflugers Arch. 356:209-222, 1975.
34. Hirche, Hj., E. Schumacher, and H. Hagemann. Extracellular K^+ concentration and K^+ balance of the gastrocnemius muscle of the dog during exercise. Pflugers Arch. 387:231-237, 1980.
35. Hirche, Hj., U. Wacker, and H. D. Langohr. Lactic acid formation in the working gastrocnemius of the dog. Int. Z. angew. Physiol. 30:52-64, 1971.
36. Hofmann, W. W. Oxygen consumption by human and rodent striated muscle in vitro. Am. J. Physiol. 230:34-40, 1976.
37. Honig, C. R., J. L. Frierson, and J. L. Patterson. Comparison of neural controls of resistance and capillary density in resting muscle. Am. J. Physiol. 218:937-942, 1970.
38. Honig, C. R., J. L. Frierson, and C. N. Nelson. O_2 transport and VO_2 in resting muscle: significance for tissue-capillary exchange. Am. J. Physiol. 220:357-363, 1971.
39. Horecker, B. L., E. Melloni, and S. Pontremoli. Fructose 1,6-bisphosphatase: Properties of the neutral enzyme and its modification by proteolytic enzymes. Advances in Enzymology and Related Areas of Molecular Biology. 42:193-227, 1975.

40. Howley, E. T. Intramuscular Fatty Acid Mobilization in Isolated, In Situ, Dog Skeletal Muscle. Doctoral dissertation. Madison, WI: The University of Wisconsin, 1969.
41. Huckabee, W. E. Relationships of pyruvate and lactate during anaerobic metabolism. I. Effects of infusion of pyruvate or glucose and of hyperventilation. J. Clin. Invest. 37:244-254, 1958.
42. Hughes, R. L., R. T. Mathie, W. Fitch, and D. Campbell. Liver blood flow and oxygen consumption during hypocapnia and IPPV in the greyhound. J. Appl. Physiol.: Respirat. Environ. Exercise Physiol. 47:290-295, 1979.
43. Hultman, E., and K. Sahlin. Acid-base balance during exercise. In: R. S. Hutton and D. I. Miller (Eds.), Exercise and Sports Sciences Reviews, Vol. 8, Franklin Inst. Press, 1980, p. 41-128.
44. Johnson, E. E. Splanchnic hemodynamic response to passive hyperventilation. J. Appl. Physiol. 38:156-162, 1975.
45. Karetzky, M. S., and S. M. Cain. Oxygen uptake stimulation following Na-L-lactate infusion in anesthetized dogs. Am. J. Physiol. 216:1486-1490, 1969.
46. Karetzky, M. S., and S. M. Cain. Effect of sodium pyruvate infusion on acid-base balance and gas exchange in the dog. Am. J. Physiol. 217:1472-1476, 1969.
47. Karetzky, M. S., and S. M. Cain. Effect of carbon dioxide on oxygen uptake during hyperventilation in normal man. J. Appl. Physiol. 28:8-12, 1970.
48. Khambatta, H. J., and S. F. Sullivan. Effects of respiratory alkalosis on oxygen consumption and oxygenation. Anesthesiology. 38:53-58, 1973.
49. Khambatta, H. J., and S. F. Sullivan. Carbon dioxide production and washout during passive hyperventilation alkalosis. J. Appl. Physiol. 37:665-669, 1974.
50. Kontos, H. A., H. P. Mauck, Jr., D. W. Richardson, and J. L. Patterson, Jr. Circulatory responses to hypocapnia in the anesthetized dog. Am. J. Physiol. 208:139-143, 1965.
51. Lucas, A., A. Thermanias, and M. Tanche. Maximum oxygen consumption in dogs during muscular exercise and cold exposure. Pflugers Arch. 388:83-87, 1980.
52. Mainwood, G. W., and P. Worsley-Brown. The effects of extracellular pH and buffer concentration on the efflux of lactate from frog sartorius muscle. J. Physiol. 250:1-22, 1975.

53. Mansour, T. E. Studies on heart phosphofructokinase: purification, inhibition, and activation. J. Biol. Chem. 238:2285-2292, 1963.
54. Masoro, E. J., and P. D. Siegal. Acid-Base Regulation: Its Physiology and Pathophysiology, Philadelphia: W. B. Saunders Co., 1977, p. 6-10.
55. Maxwell, L. C., J. K. Barclay, D. E. Mohrman, and J. A. Faulkner. Physiological characteristics of skeletal muscles of dogs and cats. Am. J. Physiol. 233:C14-C18, 1977.
56. McLane, J. A., and J. O. Holloszy. Glycogen synthesis from lactate in the three types of skeletal muscle. J. Biol. Chem. 254:6548-6553, 1979.
57. McLeod, J. J. R., and D. H. Hoover. Lactic acid production in the blood following the injection of alkalotic solutions of dextrose or of alkaline solutions alone. Am. J. Physiol. 42:460-468, 1917.
58. Mithoefer, J. C., M. S. Karetzky, and W. F. Porter. Effect of intravenous NaHCO_3 on ventilation and gas exchange in normal man. Resp. Physiol. 4:132-140, 1968.
59. Mommaerts, W. F. H. M. Metabolism and Body Temperature. In: G. Ross (Ed.), Essentials of Human Physiology. Chicago: Year Book, 1978, p. 451.
60. Nahas, G. G., J. C. Ligou, and B. Mehlman. Effects of pH changes on O_2 uptake and plasma catecholamine levels in the dog. Am. J. Physiol. 198:60-66, 1960.
61. Nahas, G. G., and C. Poyart. Effect of arterial pH alterations on metabolic activity of norepinephrine. Am. J. Physiol. 212:765-772, 1967.
62. Newsholme, E. A. Substrate cycles: Their metabolic, energetic, and thermic consequences in man. Biochem. Soc. Symp. 43:183-205, 1978.
63. Newsholme, E. A. A possible metabolic basis for the control of body weight. New Engl. J. Med. 302:400-405, 1980.
64. Newsholme, E. A., and B. Crabtree. Substrate cycles in metabolic regulation and in heat generation. Biochem. Soc. Symp. 41:61-109, 1976.

65. Newsholme, E. A., and N. L. Jones. Intermediary Metabolism. In: Hypoxia: Man at Altitude, Edited by J. R. Sutton, N. L. Jones, and C. S. Houston. New York: Thieme-Stratton Inc., 1982, p. 29-37.
66. Newsholme, E. A., and A. H. Underwood. The control of glycolysis and gluconeogenesis in the kidney cortex. Biochem. J. 99:24C-26C, 1966.
67. Otis, A. B. Quantitative relationships in steady-state gas exchange. In: Handbook of Physiology. Respiration. Washington, DC: Am. Physiol. Soc., sect. 3, Vol. I, chapt. 27, 1964, p. 681-698.
68. Pappenheimer, J. R. Vasoconstrictor nerves and oxygen consumption in the isolated perfused hindlimb muscles of the dog. J. Physiol. 99:182-200, 1941.
69. Pappenheimer, J. R. Blood flow, arterial oxygen saturation, and oxygen consumption in the isolated perfused hindlimb of the dog. J. Physiol. 99:283-303, 1941.
70. Passon, P. G., and J. D. Peuler. A simplified radiometric assay for plasma norepinephrine and epinephrine. Anal. Biochem. 51:618-631, 1973.
71. Patterson, R. W., and S. F. Sullivan. Determinants of oxygen uptake during sodium bicarbonate infusion. J. Appl. Physiol.: Respirat. Environ. Exercise Physiol. 45:399-402, 1978.
72. Peronnet, F., R. A. Nadeau, J. D. Champlain, P. Magrassi, and C. Chatrand. Exercise plasma catecholamines in dogs: role of adrenals and cardiac nerve endings. Am. J. Physiol. 241:H243-H247, 1981.
73. Purich, D. L., H. J. Fromm, and F. B. Rudolph. The hexokinases: kinetic, physical, and regulatory properties. Advances in Enzymology and Related Areas of Molecular Biology. 39:249-326, 1973.
74. Riggs, T. E., A. W. Shafer, and C. A. Guenter. Physiologic effects of passive hyperventilation on oxygen delivery and consumption. Proc. Soc. Exp. Biol. Med. 140:1414-1417, 1972.
75. Rossing, R. G., and S. M. Cain. A nomogram relating PO_2 , pH, temperature, and hemoglobin saturation in the dog. J. Appl. Physiol. 21:195-201, 1966.
76. Schmitt, M., P. Meunier, A. Rochas, and J. Chatonnet. Catecholamines and oxygen uptake in dog skeletal muscle in situ. Pflugers Arch. 345:145-158, 1973.

77. Scholander, P. F. Analyzer for accurate estimation of respiratory gases in one-half cubic centimeter samples. J. Biol. Chem. 167:235-250, 1947.
78. Spaich, P., W. Usinger, and C. Albers. Oxygen cost of panting in anesthetized dogs. Resp. Physiol. 5:302-314, 1968.
79. Stainsby, W. N., J. T. Fales, and J. L. Lilienthal, Jr. Effect of stretch on oxygen consumption of dog skeletal muscle in situ. Bull. Johns Hopkins Hosp. 99:249-261, 1956.
80. Stainsby, W. N., and A. B. Otis. Blood flow, blood oxygen tension, oxygen uptake, and oxygen transport in skeletal muscle. Am. J. Physiol. 206:858-866, 1964.
81. Stainsby, W. N., and H. G. Welch. Lactate metabolism of contracting dog skeletal muscle in situ. Am. J. Physiol. 211:177-183, 1966.
82. Stewart, P. A. Modern quantitative acid-base chemistry. Can. J. Physiol. Pharmacol. 61:1442-1461, 1983.
83. Theye, R. A., A. L. Brown, and R. A. Van Dyke. The effects of halothane and succinylcholine on oxygen uptake of the canine gracilis muscle. Anesthesiology. 35:304-308, 1971.
84. Theye, R. A., G. A. Gronert, and J. J. A. Heffron. Oxygen uptake of canine whole body and hind limb with hypocapnic alkalosis. Anesthesiology. 47:416-422, 1977.
85. Tobin, R. B., and M. A. Mehlman. pH effects on O₂ consumption and on lactate and pyruvate production by liver slices. Am. J. Physiol. 221:1151-1155, 1971.
86. Trivedi, B., and W. H. Danforth. Effect of pH on the kinetics of frog muscle phosphofructokinase. J. Biol. Chem. 241:4110-4114, 1966.
87. Ui, M. A role of phosphofructokinase in pH-dependent regulation of glycolysis. Biochim. Biophys. Acta. 124:310-322, 1966.
88. Van Slyke, D. D., and J. M. Neill. The determination of gasses in blood and other solutions by vacuum extraction and manometric measurement. J. Biol. Chem. 61:523-573, 1924.
89. Viires, N., G. Sillye, M. Aubier, A. Rassidakis, and Ch. Roussos. Regional blood flow distribution in dog during induced hypotension and low cardiac output. J. Clin. Invest. 72:935-947, 1983.

90. Welch, H. G., and W. N. Stainsby. Oxygen debt in contracting dog skeletal muscle in situ. Resp. Physiol. 3:229-242, 1967.
91. Yhap, E. O., C. B. Wright, N. A. Popovic, and E. C. Alix. Decreased oxygen uptake with stored blood in the isolated hindlimb. J. Appl. Physiol. 38:882-885, 1975.
92. Zborowska-Sluis, D. T., and J. B. Dossetor. Hyperlactatemia of hyperventilation. J. Appl. Physiol. 22:746-755, 1967.

APPENDIX

Table A-1. Individual data for intact hind limb experiments.

	n	Control	Alkalosis
Dog 1 (7.7 kg) C-HV			
V _{O2} (ml/min)	4	1.0 ± 0.1	1.3 ± 0.1
[Lactate] _a (mM)	2	3.2 ± 0.1	5.7 ± 0.2
Flow (ml/min)	4	12.8 ± 0.4	9.1 ± 0.5
CaO ₂ (ml/100 ml)	4	24.1 ± 0.1	24.4 ± 0.2
CvO ₂ (ml/100 ml)	4	12.4 ± 0.8	13.6 ± 0.8
a-v O ₂ (ml/100 ml)	4	7.8 ± 0.2	14.1 ± 0.6
[H ⁺] _a (nM)	4	45 ± 1	29 ± 0
PaCO ₂ (Torr)	4	30 ± 0	10 ± 0
[HCO ₃ ⁻] _a (mM)	4	6 ± 1	8 ± 0
PaO ₂ (Torr)	4	92 ± 0	110 ± 1
MABP (mm Hg)	4	152 ± 3	114 ± 2
Esoph Temp (°C)	4	36.0 ± 0.0	35.0 ± 0.0
Hematocrit	2	52 ± 1	51 ± 1
Dog 2 (28.1 kg) HV-C			
V _{O2} (ml/min)	4	3.0 ± 0.1	2.7 ± 0.3
[Lactate] _a (mM)	2	2.1 ± 0.1	2.9 ± 0.2
Flow (ml/min)	4	57.4 ± 6.9	49.8 ± 3.5
CaO ₂ (ml/100 ml)	4	17.8 ± 0.12	19.2 ± 0.2
CvO ₂ (ml/100 ml)	4	12.4 ± 0.81	13.6 ± 0.8
a-v O ₂ (ml/100 ml)	4	5.4 ± 0.77	5.5 ± 0.8
[H ⁺] _a (nM)	4	43 ± 0	25 ± 1
PaCO ₂ (Torr)	4	28 ± 0	12 ± 0
[HCO ₃ ⁻] _a (mM)	4	16 ± 0	12 ± 0
PaO ₂ (Torr)	4	69 ± 2	69 ± 4
MABP (mm Hg)	4	115 ± 5	111 ± 8
Esoph Temp (°C)	4	36.9 ± 0.0	36.7 ± 0.1
Hematocrit	2	41 ± 1	41 ± 1
Dog 3 (17.2 kg) C-HV			
V _{O2} (ml/min)	4	0.9 ± 0.2	1.4 ± 0.5
[Lactate] _a (mM)	2	1.7 ± 0.1	3.4 ± 0.4
Flow (ml/min)	4	62.5 ± 1.4	28.2 ± 2.9
CaO ₂ (ml/100 ml)	4	18.0 ± 0.2	17.8 ± 0.2
CvO ₂ (ml/100 ml)	4	16.7 ± 0.2	12.9 ± 0.6
a-v O ₂ (ml/100 ml)	4	1.4 ± 0.4	4.9 ± 1.6

Table A-1 (Continued)

	n	Control	Alkalosis
[H ⁺] _a (nM)	4	46 ± 1	22 ± 0
PaCO ₂ (Torr)	4	39 ± 1	19 ± 1
[HCO ₃ ⁻] _a (mM)	4	20 ± 0	21 ± 1
PaO ₂ (Torr)	4	63 ± 2	69 ± 2
MABP (mm Hg)	4	114 ± 4	74 ± 3
Esoph Temp (°C)	4	36.5 ± 0.2	35.3 ± 0.1
Hematocrit	2	43 ± 0	39 ± 1
Dog 4 (19.1 kg) HV-C			
V _{O2} (ml/min)	4	1.4 ± 0.3	2.0 ± 0.1
[Lactate] _a (mM)	2	1.4 ± 0.1	2.5 ± 0.0
Flow (ml/min)	4	30.6 ± 1.5	28.0 ± 1.5
CaO ₂ (ml/100 ml)	4	15.6 ± 0.1	16.9 ± 0.4
CvO ₂ (ml/100 ml)	4	11.1 ± 0.6	9.7 ± 0.3
a-v O ₂ (ml/100 ml)	4	4.5 ± 0.7	7.2 ± 0.6
[H ⁺] _a (nM)	4	41 ± 1	24 ± 0
PaCO ₂ (Torr)	4	33 ± 4	16 ± 1
[HCO ₃ ⁻] _a (mM)	4	19 ± 2	16 ± 1
PaO ₂ (Torr)	4	66 ± 2	54 ± 1
MABP (mm Hg)	4	160 ± 1	174 ± 3
Esoph Temp (°C)	4	35.9 ± 0.1	35.8 ± 0.1
Hematocrit	2	35 ± 0	36 ± 1
Dog 5 (34.0 kg) C-HV			
V _{O2} (ml/min)	4	2.4 ± 0.2	2.7 ± 0.1
[Lactate] _a (mM)	2	1.4 ± 0.0	2.2 ± 0.1
Flow (ml/min)	4	57.4 ± 3.1	47.2 ± 4.1
CaO ₂ (ml/100 ml)	4	22.9 ± 0.3	23.4 ± 0.2
CvO ₂ (ml/100 ml)	4	18.6 ± 0.2	17.7 ± 0.2
a-v O ₂ (ml/100 ml)	4	4.3 ± 0.3	5.7 ± 0.1
[H ⁺] _a (nM)	4	46 ± 1	27 ± 0
PaCO ₂ (Torr)	4	40 ± 4	17 ± 1
[HCO ₃ ⁻] _a (mM)	4	21 ± 1	15 ± 1
PaO ₂ (Torr)	4	71 ± 1	76 ± 2
MABP (mm Hg)	4	157 ± 1	142 ± 3
Esoph Temp (°C)	4	37.8 ± 0.1	36.6 ± 0.1
Hematocrit	2	51 ± 0	49 ± 0

Table A-1 (Continued)

	n	Control	Alkalosis
Dog 6 (13.2 kg) HV-C			
V _{O2} (ml/min)	4	0.7 ± 0.1	0.9 ± 0.1
[Lactate] _a (mM)	2	1.6 ± 0	2.2 ± 0.2
Flow (ml/min)	4	23.7 ± 1.6	21.0 ± 0.8
CaO ₂ (ml/100 ml)	4	20.7 ± 0.2	23.3 ± 0.0
CvO ₂ (ml/100 ml)	4	17.6 ± 0.3	18.9 ± 0.3
a-v O ₂ (ml/100 ml)	4	3.1 ± 0.1	4.4 ± 0.3
[H ⁺] _a (nM)	4	46 ± 1	26 ± 0
PaCO ₂ (Torr)	4	39 ± 2	18 ± 0
[HCO ₃ ⁻] _a (mM)	4	21 ± 0	16 ± 0
PaO ₂ (Torr)	4	73 ± 1	94 ± 1
MABP (mm Hg)	4	130 ± 1	115 ± 4
Esoph Temp (°C)	4	37.2 ± 0.1	37.9 ± 0.1
Hematocrit	2	48 ± 0	50 ± 0
Dog 7 (25.0 kg) C-HV			
V _{O2} (ml/min)	4	2.2 ± 0.2	2.6 ± 0.1
[Lactate] _a (mM)	2	1.5 ± 0.2	2.2 ± 0.2
Flow (ml/min)	4	47.2 ± 1.0	24.9 ± 2.2
CaO ₂ (ml/100 ml)	4	20.1 ± 0.2	20.9 ± 0.3
CvO ₂ (ml/100 ml)	4	15.5 ± 0.5	10.2 ± 1.0
a-v O ₂ (ml/100 ml)	4	4.6 ± 0.5	10.7 ± 0.8
[H ⁺] _a (nM)	4	41 ± 0	25 ± 0
PaCO ₂ (Torr)	4	31 ± 1	14 ± 0
[HCO ₃ ⁻] _a (mM)	4	18 ± 0	14 ± 0
PaO ₂ (Torr)	4	59 ± 2	72 ± 1
MABP (mm Hg)	4	125 ± 3	99 ± 1
Esoph Temp (°C)	4	39.5 ± 0.0	38.9 ± 0.1
Hematocrit	2	48 ± 1	45 ± 1
Dog 8 (17.2 kg) HV-C			
V _{O2} (ml/min)	4	1.2 ± 0.0	1.4 ± 0.0
[Lactate] _a (mM)	2	2.1 ± 0.2	4.9 ± 0.0
Flow (ml/min)	4	18.0 ± 0.2	19.4 ± 1.5
CaO ₂ (ml/100 ml)	4	14.6 ± 0.1	16.9 ± 0.2
CvO ₂ (ml/100 ml)	4	7.8 ± 0.1	9.6 ± 0.2
a-v O ₂ (ml/100 ml)	4	6.8 ± 0.1	7.3 ± 0.6

Table A-1 (Continued)

	n	Control	Alkalosis
[H ⁺]a (nM)	4	43 ± 0	25 ± 0
PaCO ₂ (Torr)	4	35 ± 0	13 ± 0
[HCO ₃ ⁻]a (mM)	4	19 ± 0	12 ± 0
PaO ₂ (Torr)	4	72 ± 0	92 ± 0
MABP (mm Hg)	4	74 ± 2	52 ± 2
Esoph Temp (°C)	4	38.4 ± 0.1	38.0 ± 0.0
Hematocrit	4	34 ± 1	36 ± 1

Values are means ± SE. Value for n is the same for both the control and alkalosis treatments. Dog weight and order of experimental treatments are indicated. C = Control, HV = Hyperventilation

Table A-2. Individual data for tied hind limb experiments.

	n	Control	Alkalosis
Dog 1 (15.9 kg, 644 g) C-HV			
V02 (ml/min)	9	3.1 ± 0.1	3.4 ± 0.1
V02 (ml/kg per min)	9	4.8 ± 0.2	5.2 ± 0.2
[Lactate] _a (mM)	3	2.2 ± 0.1	3.4 ± 0.3
Flow (ml/min)	9	56.2 ± 1.0	64.8 ± 0.8
Flow (ml/100 g min)	9	8.7 ± 0.2	10.1 ± 0.1
CaO ₂ (ml/100 ml)	9	21.8 ± 0.2	21.9 ± 0.1
CvO ₂ (ml/100 ml)	9	16.3 ± 0.3	16.7 ± 0.2
a-v O ₂ (ml/100 ml)	9	5.5 ± 0.2	5.2 ± 0.2
[H ⁺] _a (nM)	5	47 ± 0	27 ± 1
[H ⁺] _v (nM)	4	52 ± 2	31 ± 1
PaCO ₂ (Torr)	5	33 ± 1	14 ± 0
PvCO ₂ (Torr)	4	38 ± 2	17 ± 1
[HCO ₃ ⁻] _a (mM)	5	17 ± 0	12 ± 0
[HCO ₃ ⁻] _v (mM)	4	18 ± 1	13 ± 1
PaO ₂ (Torr)	5	85 ± 1	108 ± 1
PvO ₂ (Torr)	4	41 ± 1	36 ± 1
MABP (mm Hg)	5	131 ± 5	115 ± 1
Esoph Temp (°C)	5	36.3 ± 0.1	33.7 ± 0.1
Hematocrit	3	48 ± 0	45 ± 0
Dog 2 (14.5 kg, 275 g) HV-C			
V02 (ml/min)	9	2.2 ± 0.2	2.0 ± 0.1
V02 (ml/kg per min)	9	7.8 ± 0.6	7.2 ± 0.4
[Lactate] _a (mM)	3	2.6 ± 0.2	3.7 ± 0.2
Flow (ml/min)	9	49.3 ± 2.8	40.3 ± 1.0
Flow (ml/100 g min)	9	17.9 ± 1.0	14.6 ± 0.4
CaO ₂ (ml/100 ml)	9	17.5 ± 0.1	18.3 ± 0.1
CvO ₂ (ml/100 ml)	9	13.0 ± 0.4	13.2 ± 0.4
a-v O ₂ (ml/100 ml)	9	4.4 ± 0.3	5.1 ± 0.4
[H ⁺] _a (nM)	5	40 ± 1	21 ± 0
[H ⁺] _v (nM)	4	42 ± 1	25 ± 0
PaCO ₂ (Torr)	5	31 ± 1	10 ± 0
PvCO ₂ (Torr)	4	36 ± 1	14 ± 0
[HCO ₃ ⁻] _a (mM)	5	19 ± 0	11 ± 0
[HCO ₃ ⁻] _v (mM)	4	20 ± 0	14 ± 0
PaO ₂ (Torr)	5	69 ± 1	76 ± 1
PvO ₂ (Torr)	4	37 ± 2	22 ± 2
MABP (mm Hg)	5	93 ± 0	81 ± 7
Esoph Temp (°C)	5	36.3 ± 0.0	35.0 ± 0.1
Hematocrit	3	38 ± 0	38 ± 0

Table A-2 (Continued)

	n	Control	Alkalosis
Dog 3 (7.7 kg, 271 g) C-HV			
V02 (ml/min)	9	2.1 ± 0.1	1.8 ± 0.0
V02 (ml/kg per min)	9	7.6 ± 0.3	6.6 ± 0.2
[Lactate]a (mM)	3	1.5 ± 0.2	4.3 ± 0.6
Flow (ml/min)	9	14.7 ± 0.6	12.7 ± 0.3
Flow (ml/100 g min)	9	5.4 ± 0.2	4.7 ± 0.1
CaO2 (ml/100 ml)	9	20.7 ± 0.1	19.87 ± 0.06
CvO2 (ml/100 ml)	9	6.7 ± 0.3	5.80 ± 0.20
a-v O2 (ml/100 ml)	9	14.0 ± 0.4	14.07 ± 0.20
[H+]a (nM)	5	47 ± 0	30 ± 0
[H+]v (nM)	4	53 ± 1	48 ± 1
PaCO2 (Torr)	5	32 ± 1	12 ± 1
PvCO2 (Torr)	4	47 ± 2	34 ± 2
[HCO3 ⁻]a (mM)	5	16 ± 0	9 ± 0
[HCO3 ⁻]v (mM)	4	21 ± 1	17 ± 1
PaO2 (Torr)	5	71 ± 1	110 ± 1
PvO2 (Torr)	4	21 ± 2	30 ± 1
MABP (mm Hg)	5	93 ± 5	77 ± 2
Esoph Temp (°C)	5	37.8 ± 0.2	36.7 ± 0.1
Hematocrit	3	46 ± 1	42 ± 0
Dog 4 (18.1 kg, 445 g) HV-C			
V02 (ml/min)	9	3.1 ± 0.2	2.9 ± 0.12
V02 (ml/kg per min)	9	7.0 ± 0.4	6.6 ± 0.26
[Lactate]a (mM)	3	1.4 ± 0.4	3.4 ± 0.2
Flow (ml/min)	9	42.1 ± 0.5	44.2 ± 1.2
Flow (ml/100 g min)	9	9.5 ± 0.1	10.0 ± 0.3
CaO2 (ml/100 ml)	9	19.4 ± 0.1	22.8 ± 0.1
CvO2 (ml/100 ml)	9	12.0 ± 0.4	16.1 ± 0.4
a-v O2 (ml/100 ml)	9	7.4 ± 0.3	6.7 ± 0.4
[H+]a (nM)	5	47 ± 1	26 ± 1
[H+]v (nM)	4	50 ± 1	32 ± 1
PaCO2 (Torr)	5	32 ± 2	12 ± 1
PvCO2 (Torr)	4	41 ± 1	19 ± 0
[HCO3 ⁻]a (mM)	5	17 ± 1	11 ± 0
[HCO3 ⁻]v (nM)	4	20 ± 0	14 ± 0
PaO2 (Torr)	5	60 ± 2	76 ± 1
PvO2 (Torr)	4	36 ± 2	34 ± 2
MABP (mm Hg)	5	114 ± 1	91 ± 3
Esoph Temp (°C)	5	37.9 ± 0.0	37.2 ± 0.1
Hematocrit	3	48 ± 0	48 ± 0

Table A-2 (Continued)

	n	Control	Alkalosis
Dog 5 (15.9 kg, 595 g) C-HV			
V02 (ml/min)	9	2.7 ± 0.1	2.7 ± 0.1
V02 (ml/kg per min)	9	4.5 ± 0.2	4.6 ± 0.2
[Lactate] _a (mM)	3	1.5 ± 0.1	2.7 ± 0.4
Flow (ml/min)	9	40.0 ± 1.4	28.0 ± 0.6
Flow (ml/100 g min)	9	6.7 ± 0.2	4.7 ± 0.1
CaO ₂ (ml/100 ml)	9	21.3 ± 0.2	22.1 ± 0.1
CvO ₂ (ml/100 ml)	9	14.6 ± 0.3	12.4 ± 0.3
a-v O ₂ (ml/100 ml)	9	6.7 ± 0.3	9.7 ± 0.3
[H ⁺] _a (nM)	5	42 ± 0	24 ± 0
[H ⁺] _v (nM)	4	46 ± 0	31 ± 0
PaCO ₂ (Torr)	5	31 ± 0	11 ± 0
PvCO ₂ (Torr)	4	38 ± 1	21 ± 1
[HCO ₃ ⁻] _a (mM)	5	18 ± 0	11 ± 0
[HCO ₃ ⁻] _v (mM)	4	20 ± 1	17 ± 0
PaO ₂ (Torr)	5	68 ± 1	100 ± 1
PvO ₂ (Torr)	4	36 ± 1	30 ± 2
MABP (mm Hg)	5	121 ± 4	82 ± 1
Esoph Temp (°C)	5	38.6 ± 0.1	36.6 ± 0.1
Hematocrit	3	48 ± 1	45 ± 1

Values are means ± SE. Value for n is the same for both the control and alkalosis treatments. Dog weight, hind limb muscle wt, and order of treatments are indicated for each experiment. C = Control, HV = Hyperventilation

Table A-3. Data from individual gracilis experiments.

	n	Control	Alkalosis
Dog 1 (18.1 kg, 59 g) C-HV			
V02 (ml/kg per min)	7	8.4 ± 0.4	7.1 ± 0.4
WB V02 (ml/kg per min)	2	6.1 ± 0.3	-
[Lactate]a (mM)	2	4.1 ± 0.0	6.2 ± 0.5
[Lactate]v (mM)	2	4.4 ± 0.1	7.1 ± 0.6
Flow (ml/100 g min)	7	10.8 ± 0.2	6.6 ± 0.5
CaO2 (ml/100 ml)	7	21.8 ± 0.1	21.9 ± 0.1
CvO2 (ml/100 ml)	7	14.1 ± 0.4	10.9 ± 0.3
a-v O2 (ml/100 ml)	7	7.7 ± 0.4	11.0 ± 0.2
[H+]a (nM)	4	42 ± 1	30 ± 0
[H+]v (nM)	3	49 ± 0	42 ± 0
PaCO2 (Torr)	4	30 ± 0	13 ± 0
PvCO2 (Torr)	3	40 ± 0	29 ± 2
[HCO3 ⁻]a (mM)	4	18 ± 0	11 ± 0
[HCO3 ⁻]v (mM)	3	19 ± 0	17 ± 1
MABP (mm Hg)	3	104 ± 2	78 ± 0
Muscle Temp (°C)	3	37.7 ± 0.3	37.9 ± 0.1
Esoph Temp (°C)	3	37.2 ± 0.0	36.3 ± 0.0
Hematocrit	2	47 ± 1	45 ± 0
Dog 2 (22.7 kg, 64 g) C-HV			
V02 (ml/kg per min)	7	9.1 ± 0.4	8.1 ± 0.4
WB V02 (ml/kg per min)	2	5.4 ± 0.1	-
[Lactate]a (mM)	2	4.0 ± 0.2	5.0 ± 0.0
[Lactate]v (mM)	2	4.4 ± 0.0	5.4 ± 0.1
Flow (ml/100 g min)	7	7.8 ± 0.2	6.2 ± 0.4
CaO2 (ml/100 ml)	7	19.4 ± 0.2	22.9 ± 0.1
CvO2 (ml/100 ml)	7	7.7 ± 0.3	9.7 ± 0.4
a-v O2 (ml/100 ml)	7	11.7 ± 0.5	13.2 ± 0.3
[H+]a (nM)	4	47 ± 1	26 ± 0
[H+]v (nM)	3	51 ± 2	34 ± 1
PaCO2 (Torr)	4	35 ± 1	11 ± 0
PvCO2 (Torr)	3	39 ± 3	24 ± 0
[HCO3 ⁻]a (mM)	4	18 ± 0	11 ± 0
[HCO3 ⁻]v (mM)	3	18 ± 1	17 ± 0
MABP (mm Hg)	3	99 ± 2	100 ± 2
Muscle Temp (°C)	3	37.6 ± 0.2	37.7 ± 0.1
Esoph Temp (°C)	3	38.1 ± 0.1	36.5 ± 0.1
Hematocrit	2	45 ± 2	46 ± 0

Table A-3 (Continued)

	n	Control	Alkalosis
Dog 3 (24.6 kg, 87 g) C-HV			
V02 (ml/kg per min)	7	9.9 ± 0.3	7.2 ± 0.6
WB V02 (ml/kg per min)	2	6.5 ± 0.1	-
[Lactate]a (mM)	2	1.4 ± 0.6	3.3 ± 0.2
[Lactate]v (mM)	2	1.5 ± 0.5	4.1 ± 0.5
Flow (ml/10 g min)	7	13.6 ± 0.4	7.3 ± 0.8
CaO2 (ml/100 ml)	7	18.6 ± 0.1	19.2 ± 0.1
CvO2 (ml/100 ml)	7	11.4 ± 0.2	9.1 ± 0.5
a-v O2 (ml/100 ml)	7	7.2 ± 0.2	10.1 ± 0.4
[H+]a (nM)	4	44 ± 0	30 ± 0
[H+]v (nM)	3	49 ± 2	42 ± 1
PaCO2 (Torr)	4	34 ± 1	18 ± 0
PvCO2 (Torr)	3	39 ± 0	29 ± 0
[HCO3 ⁻]a (mM)	4	19 ± 0	14 ± 0
[HCO3 ⁻]v (mM)	3	20 ± 1	17 ± 1
MABP (mm Hg)	3	97 ± 2	58 ± 1
Muscle Temp (°C)	3	38.1 ± 0.1	37.9 ± 0.5
Esoph Temp (°C)	3	37.4 ± 0.1	36.6 ± 0.2
Hematocrit	2	40 ± 1	39 ± 1

WB V02 refers to whole-body O2 uptake. Values are means ± SE.
 Value for n is the same for both treatments. Dog wt, gracilis muscle
 wt, and order of treatment are indicated. C = Control,
 HV = Hyperventilation.

Table A-4. Individual data for gastrocnemius experiments.

	n	Control	Alkalosis
Dog 1 (- kg, 71.4 g) C-HV			
VO2 (ml/kg per min)	7	3.7 ± 0.3	4.5 ± 0.2
[Lactate]a (mM)	2	0.6 ± 0.0	1.4 ± 0.7
[Lactate]v (mM)	2	0.6 ± 0.1	1.8 ± 1
Flow (ml/100 g min)	7	11.1 ± 0.5	4.9 ± 0.6
CaO2 (ml/100 ml)	7	22.9 ± 0.1	23.9 ± 0.1
CvO2 (ml/100 ml)	7	19.5 ± 0.3	14.0 ± 1.3
a-v O2 (ml/100 ml)	7	3.4 ± 0.4	9.9 ± 1.1
[H+]a (nM)	4	45 ± 1	24 ± 0
[H+]v (nM)	3	46 ± 0	28 ± 0
PaCO2 (Torr)	4	33 ± 3	12 ± 0
PvCO2 (Torr)	3	39 ± 1	16 ± 1
[HCO3 ⁻]a (mM)	4	18 ± 1	12 ± 1
[HCO3 ⁻]v (mM)	3	20 ± 1	14 ± 1
PaO2 (Torr)	4	72 ± 7	65 ± 1
PvO2 (Torr)	3	46 ± 1	27 ± 3
MABP (mm Hg)	3	161 ± 1	112 ± 12
Muscle Temp (°C)	3	36.2 ± 0.6	35.1 ± 0.0
Esoph Temp (°C)	3	38.1 ± 0.1	37.3 ± 0.1
Hematocrit	2	53 ± 1	50 ± 1
Dog 2 (16.8 kg, 68 g) C-HV			
VO2 (ml/kg per min)	7	3.8 ± 0.2	3.9 ± 0.1
[Lactate]a (mM)	2	0.8 ± 0.2	2.0 ± 0.3
[Lactate]v (mM)	2	0.8 ± 0.1	2.3 ± 0.1
Flow (ml/100 g min)	7	10.2 ± 0.9	10.6 ± 0.5
CaO2 (ml/100 ml)	7	18.8 ± 0.1	19.7 ± 0.1
CvO2 (ml/100 ml)	7	14.9 ± 0.3	16.0 ± 0.4
a-v O2 (ml/100 ml)	7	3.9 ± 0.4	3.8 ± 0.3
[H+]a (nM)	4	44 ± 2	21 ± 0
[H+]v (nM)	3	46 ± 1	25 ± 0
PaCO2 (Torr)	4	39 ± 2	13 ± 1
PvCO2 (Torr)	3	44 ± 1	17 ± 0
[HCO3 ⁻]a (mM)	4	21 ± 0	14 ± 1
[HCO3 ⁻]v (mM)	3	22 ± 0	
PaO2 (Torr)	4	68 ± 6	78 ± 2
PvO2 (Torr)	3	45 ± 2	35 ± 2
MABP (mm Hg)	3	136 ± 2	125 ± 1
Muscle Temp (°C)	3	34.9 ± 0.1	35.6 ± 0.2
Esoph Temp (°C)	3	37.7 ± 0.1	36.5 ± 0.1
Hematocrit	2	42 ± 1	41 ± 1

Table A-4 (Continued)

	n	Control	Alkalosis
Dog 3 (15.9 kg, 72 g) C-HV			
V02 (ml/kg per min)	7	3.3 ± 0.2	3.7 ± 0.1
[Lactate]a	2	0.2 ± 0.0	0.8 ± 0.2
[Lactate]v	2	0.4 ± 0.0	1.4 ± 0.2
Flow (ml/100 g min)	7	19.5 ± 0.6	11.7 ± 0.6
CaO2 (ml/100 ml)	7	19.4 ± 0.1	19.5 ± 0.1
CvO2 (ml/100 ml)	7	17.7 ± 0.1	16.3 ± 0.2
a-v O2 (ml/100 ml)	7	1.7 ± 0.1	3.2 ± 0.2
[H+]a (nM)	4	41 ± 0	19 ± 0
[H+]v (nM)	3	41 ± 1	21 ± 0
PaCO2 (Torr)	4	39 ± 1	13 ± 0
PvCO2 (Torr)	3	42 ± 2	15 ± 0
[HCO3 ⁻]a (mM)	4	23 ± 0	17 ± 0
[HCO3 ⁻]v (mM)	3	24 ± 0	17 ± 0
PaO2 (Torr)	4	64 ± 2	65 ± 6
PvO2 (Torr)	3	46 ± 2	31 ± 1
MABP (mm Hg)	3	131 ± 4	95 ± 0
Muscle Temp (°C)	3	35.1 ± 0.1	36.1 ± 0.1
Esoph Temp (°C)	3	37.8 ± 0.1	37.4 ± 0.3
Hematocrit	2	44 ± 1	42 ± 1
Dog 4 (- kg, 75 g) C-HV			
V02 (ml/kg per min)	7	7.4 ± 0.2	5.7 ± 0.2
[Lactate]a (mM)	2	0.7 ± 0	2.5 ± 0.20
[Lactate]v (mM)	2	0.8 ± 0.0	1.9 ± 0.2
Flow (ml/100 g min)	7	19.8 ± 0.3	20.7 ± 1.2
CaO2 (ml/100 ml)	7	15.9 ± 0.1	17.5 ± 0.1
CvO2 (ml/100 ml)	7	12.2 ± 0.1	14.7 ± 0.2
a-v O2 (ml/100 ml)	7	3.7 ± 0.1	2.8 ± 0.2
[H+]a (nM)	4	45 ± 1	20 ± 0
[H+]v (nM)	3	47 ± 0	22 ± 0
PaCO2 (Torr)	4	39 ± 1	12 ± 0
PvCO2 (Torr)	3	43 ± 0	13 ± 0
[HCO3 ⁻]a (mM)	4	20 ± 0	14 ± 0
[HCO3 ⁻]v (mM)	3	22 ± 0	14 ± 0
PaO2 (Torr)	4	67 ± 3	99 ± 1
PvO2 (Torr)	3	44 ± 0	37 ± 1
MABP (mm Hg)	3	122 ± 1	106 ± 1
Muscle Temp (°C)	3	36.0 ± 0.0	35.6 ± 0.3
Esoph Temp (°C)	3	38.0 ± 0.0	36.7 ± 0.1
Hematocrit	2	37 ± 0	36 ± 1

Table A-5. Summary of results from intact hind limb pilot studies.

Dog	wt (kg)	Control	Alkalosis
1	18.1	1.7 $\pm$ 0.1 (n=2)	2.0 $\pm$ 0.4 (n=2)
2	28.6	2.4 $\pm$ 0.2 (n=2)	4.1 $\pm$ 0.4 (n=2)
3	23.6	2.1 $\pm$ 0.1 (n=3)	2.2 $\pm$ 0.1 (n=5)
4	23.6	1.7 $\pm$ 0.1 (n=4)	2.0 $\pm$ 0.2 (n=4)
Grand Mean		2.0 $\pm$ 0.2 (n=11)	2.6 $\pm$ 0.5 (n=13)

Values are means $\pm$ SE in ml O₂/min. Dog weights are indicated.

VITA

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