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THE CONTRIBUTION OF AMMONIA TO  
EXERCISE HYPERPNEA

by

Barbara Janet Cameron

B.Sc.(Biochemistry) McMaster University, 1976

M.Sc.(Kinesiology), Simon Fraser University, 1983

THESIS SUBMITTED IN PARTIAL FULFILLMENT OF  
THE REQUIREMENTS FOR THE DEGREE OF  
DOCTOR OF PHILOSOPHY

in the School  
of  
Kinesiology

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SIMON FRASER UNIVERSITY  
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ISBN 0-315-93662-8

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Dear Barbara,

Thank you for your request to include the article that we wrote for the International Journal of Sports Medicine as an Appendix in your thesis.

I am pleased to give you my permission to include the article entitled:

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Yours sincerely,

E.W. Banister

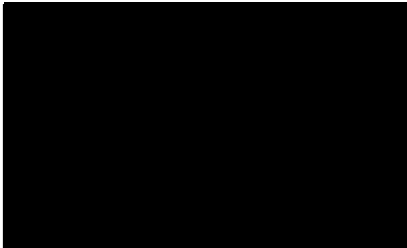
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Dear Sir,

In 1990, I collaborated in the writing of a review article for the special supplement on ammonia metabolism for the International Journal of Sports Medicine. The full citation of the article is:

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Date Approved: April 30, 92

## ABSTRACT

Many feedforward and feedback mechanisms have been proposed to account for the ventilatory response to exercise. Metabolic end-products, including elevated hydrogen ion ( $H^+$ ), potassium ion ( $K^+$ ), and catecholamine concentration, and an increase in core temperature ( $T_C$ ), are major modulating factors related to exercise hyperpnea. However, none of these potential mediators can account for the exercise-induced ventilatory response. Ammonia, which crosses the blood-brain barrier as  $NH_3$ , was investigated in this study as a potential mediator of exercise hyperpnea.

The relationship of ammonia to exercise hyperpnea was explored during two series of physical exercise: prolonged constant work rate exercise, and ramp incremental exercise to exhaustion, in either a normal glycogen, or glycogen depleted state. In the glycogen depleted subject,  $\dot{V}_E$  was significantly greater during intense, but less than maximal ramp exercise compared with the control condition, although maximum  $\dot{V}_E$  was not significantly different. Prolonged exercise at 50%  $WR_{MAX}$  in the control state resulted in an upward drift in  $\dot{V}_E$ .  $\dot{V}_E$  was also significantly greater during prolonged exercise in the glycogen depleted subject compared with the control condition. In both ramp and prolonged exercise protocols, the relative hyperventilation resulted from an increase in breathing frequency.  $V_T/T_I$ , reflecting ventilatory drive, was also significantly greater at high work rates during exercise in the glycogen depleted state compared with the control condition.

Increased sensitivity in the ventilatory system was suggested by a shift in the Euler plot ( $V_T-T_I-T_E$  diagram) in both ramp and prolonged exercise in glycogen depleted, compared with control conditions. During ramp exercise, any given mean inspiratory or expiratory flow rate ( $V_T/T_I$ , or  $V_T/T_E$ ,  $l \cdot min^{-1}$ ) was achieved by a smaller  $V_T$  and a shorter  $T_I$  or  $T_E$  in glycogen depleted subjects. In contrast, only mean expiratory flow rate was increased during prolonged exercise in glycogen depleted subjects compared with their control.



In both ramp and prolonged exercise, the pattern of  $\dot{V}_E$  could be explained, in part, by the observed change in  $[\text{NH}_3]$ . However, the contribution of  $[\text{K}^+]$ , pH,  $[\text{NE}]$ , and  $T_c$  to ventilatory drive during exercise was also recognized. pH was not a major contributor to ventilation throughout prolonged exercise in either control or glycogen depleted subjects, or during ramp exercise in glycogen depleted subjects. Maintained hyperpnea, in the absence of acidosis, supports the theory of redundancy of ventilatory control during exercise.

Although ammonia was a potential ventilatory stimulant during exercise in humans, the contribution of other mediators of ventilation could not be discounted entirely. In the glycogen depleted subject, the contribution of acidosis as a primary stimulant of ventilation was reduced, yet hyperpnea was present during exercise. There must, therefore, be redundancy in the organization of respiratory control. It is conceivable that  $\text{NH}_3$ , which is both (i) neuroactive and (ii) contributes to central neurotransmitter metabolism, may potentiate an increased sensitivity of the ventilatory response to exercise.

## DEDICATION

I would like to dedicate this thesis to Gavin,  
my husband and my best friend.

## QUOTATION

A living organism cannot be correctly studied piece by piece separately, as the parts of a machine being deduced synthetically from the separate study of each of the parts. A living organism is constantly showing itself to be a self-maintaining whole, and each part must therefore be behaving as a part of such a self-maintaining whole.

J. S. Haldane (1922)

Respiration (Oxford; University Press)

## ACKNOWLEDGEMENTS

There are a lot of people that I'd like to thank for their help and support in the preparation of this thesis. First I would like to thank Dr. Eric Banister for his encouragement and patience throughout the project. This dissertation would not have been possible without his support, and I am proud of what we have accomplished.

I would like to express my genuine appreciation to Dr. Jerome Dempsey for his willingness to evaluate this dissertation and thesis defense.

I would also like to thank Dr. Wade Parkhouse and Dr. Don McKenzie for agreeing to be members of my committee. I'm particularly grateful for the additional commitment and feedback from them leading to the completion of this dissertation. I also appreciate the contribution made by Dr. Igor Mekjavic, as Graduate Chairman, and as an examiner at the defence.

Collection of all of the data for this project would not have been possible without the able assistance of many people, and for some of them, a simple Thank You is not enough. Without Dr. Courtenay Kenny, there would have been no blood data. I'd like to thank him for his uncomplaining dedication to this project. To Dr. Tom Richardson, who became "my conscience", thank you for being patient with the subjects and our research schedule. In addition, I'd especially like to thank Mike Walsh, who contributed his programming skills to the development of the software for the data collection, and some of the complex data analysis. Francois Bellavance has also provided invaluable assistance with the statistical analysis, and in the conversion of the analysis to the University's new computer system. Ian Dunsmuir contributed much more than was required in his position as a Challenge '91 student in the lab. His sense of humour, even while moderately ketotic, relieved a lot of tension. And now we both know what it means to ASSUME!

Dr. Hugh Morton also volunteered his time willingly, and for that I am grateful. Between Hugh and Courtenay, the Southern Cross was well represented.

I would also like to thank my dedicated subjects: Ian, Peter, Rod, Stephan, Dan, and Kevin. I'm sure that you'll be reluctant to ever indulge in a ketogenic diet again.

My fellow members of the Institute's "lunch bunch" also deserve a lot of credit. Both Jim Carter and Dan Robinson have been a source of humour and tension release. May we

never again have to ask ..."is today a good day ?" Thanasis and Sanja, from "the other physiology lab" also helped me through many of the days.

Finally, there is a very special person in my life that has provided me with comfort, support, and understanding during the past few years. Gavin, you know that I would not have completed this project without you. I can't express my appreciation enough, except to say that I Love You.

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## 1.0 INTRODUCTION

"Muscular exercise is the cause of the greatest increase in rate of energy metabolism and gas exchange of the body; that breathing must therefore increase during exercise is obvious". (Dejours, 1964 ).

Ideally, the ventilatory response to exercise is appropriate and sufficient to maintain arterial blood gas and pH homeostasis. However, if the response is inadequate, respiratory acidosis would develop as a result of the increased metabolic rate and  $\text{CO}_2$  production. Conversely, excessive ventilation would produce respiratory alkalosis, characterized by a fall in  $P_{\text{ETCO}_2}$ ,  $P_{\text{aCO}_2}$ , and an increase in pH of the blood.

Many theories have been proposed to account for the change in pulmonary ventilation accompanying physical exercise, which involve feedforward or feedback stimuli. Feedforward mechanisms of respiratory stimulation include peripheral neurogenic stimulation by afferent information from peripheral motion or tension receptors, central neurogenic stimulation from cortical or hypothalamic irradiation to the respiratory centre, cardiodynamic and hemodynamic effects, metabolic rate reflected by  $\dot{V}\text{CO}_2$  or  $\dot{V}\text{O}_2$ , and blood gas oscillations. Feedback mechanisms proposed to stimulate ventilation include arterial blood gas ( $P_{\text{aO}_2}$  and  $P_{\text{aCO}_2}$ ), and hydrogen ion concentration ( $[\text{H}^+]$ ), as well as other modulating factors, for example, core temperature, plasma potassium ion and catecholamine concentration (Dempsey *et al.*, 1984; Cunningham *et al.*, 1986; Wasserman *et al.*, 1986). In addition, the existence of a yet-to-be identified humoral ventilatory mediator released in the venous effluent from exercising skeletal muscle has previously been postulated (Henderson, 1938; Asmussen and Nielsen, 1950; Dejours, 1964; Torelli and D'Angelo, 1967; Levine, 1978). Feedforward and feedback factors combine to give the net stimulus to ventilation during exercise.

The relative importance of each of these factors depends, to a certain extent, on the phase of exercise. Some appear to stimulate ventilation at the immediate onset of exercise, whereas others are effective at a higher exercise intensity, or longer duration. At the onset

of exercise, there is an abrupt increase in ventilation which occurs too rapidly to be explained by a change in the chemical constituents of the blood. It is likely that this increase is mediated by descending input from the motor cortex and the hypothalamus to the respiratory centre of the central nervous system (CNS), and by input from peripheral receptors in joints and muscles which provide information on movement which accompanies muscular contraction (Lambertson, 1974). In addition, the phase I ventilatory response is thought to be coupled to the concomitant increase in cardiac output and blood flow to the lungs at the onset of exercise (Wasserman *et al.*, 1974).

In healthy individuals, both moderate intensity, constant rate exercise and low work rate ramp incremental exercise produce an increase in pulmonary ventilation which maintains  $P_{aO_2}$ ,  $P_{aCO_2}$ , and  $[H^+]_a$  close to resting values. After the initial abrupt increase in ventilation, feedback predominantly from peripheral chemoreceptors monitoring  $P_{aCO_2}$ ,  $P_{aO_2}$ , and arterial pH seems to regulate ventilation adequately to maintain arterial blood gas and pH homeostasis.

There is obvious redundancy in the control system, in that more than one mechanism may sense the same stimulus. In the absence of one (eg. carotid bodies) a normal steady-state response may be observed, but there may be subtle changes in the dynamic response (eg. change in ventilatory kinetics) (Wasserman *et al.*, 1986).

Wasserman *et al.*, (1986) stated:

"that the control mechanism that increases ventilation in response to exercise is normally almost ideal, because it responds promptly to the increased gas-exchange rates to maintain  $P_{aCO_2}$ ,  $pH_a$ , and  $P_{aO_2}$  homeostasis during moderate exercise and responds more rapidly during heavy exercise to adjust to the added  $[H^+]$  stress "

This cannot be accepted without qualification, in relation to the control of ventilation in prolonged exercise, or during intense exercise in glycogen depleted subjects. When exercise is prolonged, there is an alteration in breathing pattern which results in a steady upward drift in pulmonary ventilation (Costill, 1970; Dempsey *et al.*, 1977; Wasserman, 1978; Martin *et al.*, 1981; Hanson *et al.*, 1982). Similarly, as the intensity increases in



ramp incremental exercise, the ventilatory response increases proportionally more than  $\dot{V}O_2$  (Wasserman and McIlroy, 1964; Wasserman *et al.*, 1973). Exercise in glycogen depleted human subjects also results in a higher ventilation at equivalent work rates, in spite of an absence of acidosis (Green *et al.*, 1979; Segal and Brooks, 1979; Jansson, 1980; Hughes *et al.*, 1982; Heigenhauser, 1983). In each instance, an accelerated metabolic demand, accumulation of metabolic end products, increased body temperature and circulating catecholamines all appear to contribute to stimulation of ventilation. However, individually, none of these potential mediators can account for every aspect of the exercise-induced ventilatory response. A question which remains to be answered is what additional factor(s) stimulate exercise hyperpnea.

The possible identification of ammonia as "an unknown substance" released in the venous effluent from muscle during exercise which may stimulate  $\dot{V}_E$  is explored in this thesis. Although it is widely accepted that acidosis is the predominant stimulus of exercise hyperpnea, at least during intense exercise, there is now sufficient contradictory evidence to suggest that this interpretation is too simplistic. Exercise hyperpnea has been dissociated from acidosis in several instances (Davis and Gass, 1981; Hagberg *et al.*, 1982; Hughes *et al.*, 1982; Farrell and Ivy, 1987), and it is apparent that acidosis is not the major contributing stimulus to the relative hyperventilation observed during prolonged exercise (Costill, 1970; Dempsey *et al.*, 1977; Wasserman, 1978; Martin *et al.*, 1981; Hanson *et al.*, 1982). In this study, the relationship of ammonia to exercise hyperpnea is explored during the period of excessive ventilation resulting from a prolonged constant work rate exercise, and ramp incremental exercise to exhaustion.

Ammonia is a normal product of metabolism, produced in large amounts in skeletal muscle during exercise. Ammonia, which is present in the plasma at a very low concentration at rest, is released into the venous effluent predominantly from active skeletal muscle during both acute exhaustive exercise, and continuous exercise of moderate intensity (Babij *et al.*, 1983; Banister *et al.*, 1983; Buono *et al.*, 1984; Dudley *et al.*, 1983;

Eriksson *et al.*, 1985; Graham *et al.*, 1987; Katz *et al.*, 1986). Thus the blood may be viewed as a vehicle for ammonia transport, giving it access to every organ in the body through normal perfusion.

Ammonia has often been correlated with an observed increase in ventilation in the absence of hypercapnia both in animals and in man (Roberts *et al.*, 1956; Hindfeldt and Siesjo, 1971; Wichser and Kazemi, 1974; Herrera, 1980 ), although to date, it has not been investigated as a respiratory stimulant during exercise.

## **REVIEW OF RELATED LITERATURE**

### **REGULATION OF BREATHING**

Ventilation is the result of a carefully synchronized pattern of inspiration and expiration. In the central nervous system, ventilation is controlled by clusters of neurons in the lower brainstem. The central respiratory centre consists primarily of the ventral respiratory group (VRG) and the dorsal respiratory group (DRG) in the medulla, and the nucleus parabrachialis medialis - Kolliker Fuse complex (pneumotaxic centre) in the rostral and lateral pons (Euler *et al.*, 1973; Cohen, 1979; Long and Duffin, 1984). The central respiratory centre processes afferent information received from chemoreceptors, baroreceptors, lung stretch receptors, and receptors in muscles and joints, and sends output to the spinal motorneurons which regulate respiratory muscle activity (Lambertson, 1974).

The DRG consists of about 95% inspiratory neurons, whereas in the VRG, about one third of the respiratory related units are expiratory neurons (Koepchen *et al.*, 1979). Almost all of the DRG inspiratory neurons project to the spinal cord, but a large number of the VRG do not have spinal cord connections. A distinct subgroup from the DRG, termed inspiratory alpha neurons, project mostly to the phrenic motorneurons, whereas the inspiratory beta neurons, which are excited by inflation, project mostly to the external

intercostal motoneurons. Inspiratory beta neurons have also been identified as inhibitory interneurons during inspiratory-to-expiratory phase switching (Cohen and Feldman, 1977; Long and Duffin, 1984). The VRG has both afferent and efferent connections to the pneumotaxic centre. The pneumotaxic centre appears to switch off, or inhibit inspiration, and thus regulate inspiratory volume and respiratory rate (Lambertson, 1974).

Separate input to the respiratory motoneurons originates from the pyramidal and extrapyramidal systems. At the spinal level, different afferent, efferent, and interneuron inputs interact to produce the final efferent output to respiratory muscles (Euler, 1983). Because of the reciprocal inhibitory interconnections between inspiratory and expiratory areas of both the medullary respiratory centres and between spinal motoneurons controlling respiratory muscle activity, neuronal discharge in the inspiratory area leads to inhibition of expiration (Lambertson, 1974).

## ANALYSIS OF BREATHING PATTERNS DURING EXERCISE

The output of the respiratory system may be analyzed in terms of pulmonary ventilation ( $\dot{V}_E$ ) and its two traditional components, tidal volume ( $V_T$ ) and ventilatory frequency ( $f$ ), as well as derived components of ventilation proposed by Milic-Emili and Grunstein (1976), inspiratory drive ( $V_T/T_I$ ) and inspiratory timing ( $T_I/T_{TOT}$ ). These relationships are described as:

$$\dot{V}_E = V_T \cdot f$$

$$\dot{V}_E = V_T/T_I \cdot T_I/T_{TOT}$$

The advantage of analyzing ventilation by the second equation is that the term  $V_T/T_I$  reflects the mean inspiratory flow rate, and is closely related to central inspiratory activity (CIA), at least in normal humans; and the other ( $T_I/T_{TOT}$ ) reflects respiratory 'timing'.

## HUMORAL FACTORS ASSOCIATED WITH THE STIMULATION OF VENTILATION

Several humoral factors are known to affect ventilation, at least at rest. Although the purpose of the study was to assess the specific relationship between ammonia and the ventilatory response to exercise in humans, it was essential to monitor other confounding factors which might also contribute substantially to exercise-induced hyperpnea.

### HYDROGEN ION CONCENTRATION

The most common humoral stimulus attributed as a primary stimulus to exercise hyperpnea is an elevated hydrogen ion concentration, ( $[H^+]$ ). (Grodins, 1950; Dejours, 1964; Holmgren and McIlroy, 1964; Comroe, 1965; Wasserman, *et al.*, 1967; Wasserman, *et al.*, 1973; Koyal, *et al.*, 1976; Wasserman, 1976; Whipp, 1977; Whipp and Davis, 1979; Beaver, 1986a; Casaburi, *et al.*, 1987). Sensitivity of the peripheral chemoreceptors to circulating  $[H^+]$  was demonstrated in artificially perfused tissue preparations by Joels and Neil (1960), and Gray (1968), who observed an increase in chemoreceptor discharge in response to decreased pH.

### METABOLIC ACIDOSIS, LACTATE, AND ASSOCIATED THRESHOLDS

Because of the stoichiometric relationship between  $[H^+]$  and lactate production in skeletal muscle, exercise-induced metabolic acidosis is frequently related to increased lactate concentration, in spite of evidence that a significant  $[H^+]$  increase during exercise more likely results from ATP hydrolysis (George and Rutman, 1960; Gevers, 1977; Zilva, 1978; Alberti and Cuthbert, 1982; Hochachka and Mommsen, 1983). Regardless of the source of  $[H^+]$ , considerable evidence supports its role as a stimulus to exercise hyperpnea. First,  $[H^+]$  is a known stimulus to ventilation at rest (Comroe, 1965) and its concentration is increased in heavy prolonged exercise. Secondly, because of the close temporal association between the ventilatory threshold (VT) and a lactate (LT) or anaerobic threshold (AT) it has been hypothesized frequently that metabolic acidosis is responsible for the

disproportionate increase in  $\dot{V}_E$  at heavy work rates (Wasserman and McIlroy, 1964; Wasserman, *et al.*, 1973; Whipp, 1977; Sutton and Jones, 1979; Wasserman, *et al.*, 1986). Thirdly, Casaburi *et al.*, (1987) reported that the reduced ventilatory response to an absolute metabolic demand, following endurance training, might be accounted for primarily by a reduced arterial acidosis, or decreased blood lactate concentration. However, in the latter study, the possibility that other metabolic changes induced by training could contribute to the reduced ventilatory response to exercise was not considered. A final observation supporting the role of  $[H^+]$  as a ventilatory stimulant during exercise is that asthmatic individuals with carotid body resection (causing elimination of peripheral sensitivity to  $[H^+]$ ), do not exhibit a hyperventilatory response to heavy exercise, although their ventilatory response to moderate steady state exercise was described as normal (Wasserman, *et al.*, 1975a). Even with carotid bodies intact, however, this patient population is unable to increase pulmonary ventilation to match demand during exercise. This must confound the interpretation of the data of Wasserman *et al.*, 1975a.

The concept that acidosis is responsible for exercise hyperpnea is controversial, and has been the subject of several recent reviews (Dempsey, *et al.*, 1985; Walsh and Banister, 1988). The contrary arguments are that:

- i) the VT may be dissociated from the AT;
- ii) exercise hyperpnea has been observed concomitant with a reduction or complete absence of a change in  $[H^+]$ ; and
- iii) low ventilation has been observed during exercise, in the presence of acidosis.

Dissociation of the VT from the AT has been shown in a variety of exercise protocols. Davis and Gass (1981) demonstrated that when two successive exercise protocols to exhaustion are separated by a 5 minute rest interval, the work rate at which VT occurs is the same in both trials, in spite of a large difference in blood lactate concentration. In the initial trial, venous lactate increased curvilinearly. During the second exercise trial, blood lactate concentration was decreasing at the point at which VT was identified. From

these data, Davis and Gass (1981) concluded that some factor other than acidosis was responsible for an increased ventilation at the VT. Farrel and Ivy (1987) confirmed these results in a study in which exercise was performed from rest, or immediately following intense interval exercise. VT occurred at a similar work rate and  $\dot{V}O_2$ , whether venous blood lactate or  $[H^+]$  was high or low. They concluded that a change in blood lactate or  $[H^+]$  could not mediate the nonlinear increase in  $\dot{V}_E$  observed during incremental exercise, and suggested that the increase in  $\dot{V}_E/\dot{V}CO_2$  corresponded with the metabolic rate of active musculature.

Hughes, *et al.* (1982) reported an uncoupling between the VT and AT during exercise with reduced muscle glycogen stores. They observed that VT and AT could be manipulated independently by varying pedalling frequency or the state of muscle glycogen depletion during cycle ergometer exercise. Increased pedalling frequency shifted the AT to a relatively lower work rate, whereas glycogen depletion elicited a shift in VT to a lower, and AT to a higher work rate relative to a normal glycogen trial.

Neary, *et al.* (1985) also concluded that an increased blood lactate was not responsible for the 'breakaway' ventilation during incremental exercise in humans. In their study of one-legged cycle ergometry to exhaustion, the group mean  $\dot{V}O_2$  at VT was unchanged in glycogen depleted subjects despite a lower exercise blood lactate concentration compared with a control condition, indicating that factors other than lactate must be responsible for the non-linear increase in ventilation.

Recently, McLellan and Gass (1989b) have challenged the dissociation of VT and LT by manipulation of exercise protocols. Their data show that LT and VT are unaffected by glycogen depletion prior to exercise, and in fact occur at a similar  $\dot{V}O_2$ .

Exercise hyperventilation has also been observed in association with a complete absence of  $[H^+]$  increase (Hagberg, *et al.*, 1982), providing further support that acidosis may not be a primary drive to ventilation. McArdle's patients demonstrate a normal hyperventilatory response during incremental exercise in the absence of an increase in

plasma  $[H^+]$ . These patients lack muscle phosphorylase and are unable to produce lactate during exercise. However it has been suggested that the ventilatory change noted in these studies could be a result of exercise-induced pain, a common symptom experienced during exercise by McArdle's syndrome patients (Whipp and Mahler, 1983). Individuals with McArdle's syndrome also hyperventilate at very light work rates, suggesting that other factors may be involved in their ventilatory drive. Overall, this patient population may not be an appropriate model to compare with healthy individuals. It is interesting that McArdle's patients also exhibit a normal slow component in their recovery ventilation after high intensity exercise (Hagberg, *et al.*, 1990). This portion of recovery ventilation is usually attributed to a "lactacid mechanism" (Margaria, *et al.*, 1933) which of course is not possible in these patients.

A hyperventilatory response to exercise has been observed in glycogen depleted, but otherwise normal subjects despite a less than normal degree of metabolic acidosis and concomitant low blood lactate concentration (Jansson, 1980; Hughes, *et al.*, 1982; Heigenhauser, *et al.*, 1983).

The blunted chemosensitivity, in response to both hypoxic and hypercapnic stimuli at rest, in endurance athletes (Martin, 1978; Schoene, 1982) and a reduced hyperventilatory response to intense exercise in well trained athletes, even in the presence of metabolic acidosis (Dempsey, 1984), provides additional evidence against acidosis as a primary ventilatory drive.

## **CARBON DIOXIDE DELIVERY TO THE LUNG**

When there is a rise in arterial  $PCO_2$  due to increased tissue metabolism, ventilation is stimulated. With an increase in the rate of pulmonary excretion of  $CO_2$ , arterial  $PCO_2$  returns to normal, and the ventilatory stimulus decreases. (For a complete review of the integration of the respiratory response to changes in alveolar partial pressures of  $CO_2$ ,  $O_2$ , and in arterial pH, see Cunningham, *et al.*, 1986). During exercise of moderate intensity,

however,  $\text{PaCO}_2$  does not increase systematically (Asmussen and Nielsen, 1958; Holmgren and McIlroy, 1964; Hansen, *et al.*, 1967; Whipp and Wasserman, 1969; Jones, 1975). It has been suggested, instead, that during exercise an increase in  $\text{CO}_2$  flux to the lung from a stimulated metabolism may be the initial and continuing signal to the ventilatory control centre to increase  $\dot{V}_E$  in proportion to the change in  $\text{CO}_2$  flux. An increase in the rate of carbon dioxide delivery to the lungs during muscular exercise results in an increase in alveolar ventilation (Comroe, 1965; Wasserman, *et al.*, 1967). Yamamoto and Edwards (1960) and Wasserman *et al.*, (1974, 1975b) suggested that  $\text{CO}_2$  flow to the lung, without an increment in mean arterial  $\text{PCO}_2$ , was responsible for the increased exercise-induced ventilation. Wasserman *et al.*, (1974) assessed the influence of increased cardiac output, resulting from either isoproterenol infusion or cardiac pacing, upon ventilation. It was concluded that an increased  $\dot{V}_E$  accompanying an increased cardiac output resulted directly from an increased carbon dioxide flux.

McLellan and Gass (1989a) concluded that increased  $\text{CO}_2$  flow to the lung was responsible for a higher absolute ventilation during exercise at the AT in a group of trained cyclists compared to control subjects. In spite of the fact that the AT exercise represented a higher absolute or relative  $\dot{V}\text{O}_2$  for cyclists compared with controls, the group mean ventilatory equivalent for carbon dioxide production ( $\dot{V}_E/\dot{V}\text{CO}_2$ ) was similar in cyclists to the response for low AT subjects.

Not all studies support the theory that an increased  $\text{CO}_2$  flux constitutes the humoral stimulus to ventilation. By comparing airway and venous  $\text{CO}_2$  loading, Lamb (1966) and Lewis (1972) concluded, independently, that an unavoidable increment in mean arterial  $\text{PCO}_2$  could account for an increased  $\dot{V}_E$  induced by venous  $\text{CO}_2$  loading.

Heigenhauser, *et al.*, (1983) also investigated the ventilatory effect of  $\text{CO}_2$  flow to the lung in humans during exercise in glycogen depleted subjects. Since glycogen depletion results in a shift towards increased fatty acid utilization by muscle, and therefore a reduction in  $\dot{V}\text{CO}_2$  and  $\text{CO}_2$  flux to the lung, it was hypothesized that a decrease in  $\dot{V}_E$



would result, if  $\text{CO}_2$  flux is an important ventilatory stimulant. Contrary to expectation,  $\dot{V}_E$  increased during exercise in glycogen depleted subjects despite a similar  $\dot{V}_{\text{CO}_2}$  production, compared with equivalent work in normal glycogen subjects. Increased  $\dot{V}_E$  during exercise in glycogen depleted subjects could not be attributed to humoral factors known to stimulate  $\dot{V}_E$ , i.e., elevated  $\text{PaCO}_2$ , or reduced  $\text{PaO}_2$  or  $\text{pH}$ . During exercise, glycogen depleted subjects had a higher  $\dot{V}_E/\dot{V}_{\text{CO}_2}$  ratio, lower end-tidal and mixed-venous  $\text{CO}_2$  partial pressures, and higher blood  $\text{pH}$  than in the control studies. In addition, a change in  $\text{CO}_2$  flux to the lungs could not explain the higher  $\dot{V}_E$  accompanying exercise in glycogen depleted subjects, suggesting that other factors modulate  $\dot{V}_E$  under these experimental conditions. Jansson (1980) reported similar findings in glycogen depleted subjects. Heigenhauser, *et al.*, (1983) proposed a "neurogenic" mechanism to account for both the higher HR and higher  $\dot{V}_E$  observed during exercise in a glycogen depleted state compared with control. This does not discount the possibility that another humoral agent was responsible for these effects.

## CATECHOLAMINES

During exercise, an increase in plasma concentration of NE and E as a result of activation of the sympathoadrenal system is important for regulation of both the circulation and general metabolism. The combined action of the sympathetic nervous system and circulating catecholamines during exercise contributes to the inotropic (Sonnenblick, 1962, 1965, 1969) and chronotropic (West, *et al.*, 1956) response of the heart, the distribution of blood flow to working muscle (Rowell, 1974; Rowell, 1986), and the mobilization of glycogen (Gollnick, *et al.*, 1970; Galbo, 1983) and lipid (Gollnick, *et al.*, 1970; Hales, *et al.*, 1978; Galbo, 1983) as fuel substrates for exercise.

The increased concentration of catecholamines in plasma during exercise has been related to the type, intensity and duration of exercise (Galbo, 1983). A recent review on catecholamines and exercise observed that the ratio of plasma NE:E in human subjects is

generally three to four at rest, and that during incremental exercise to exhaustion, the ratio is not altered (Mazzeo, 1991). It was suggested that a change in the ratio of NE:E might indicate a change in the proportion of catecholamines released from the sympathetic nervous system and adrenal glands during exercise. Other studies have shown either a decrease, or an increase in the ratio of plasma NE:E in response to an exercise stimulus in humans. The ratio of plasma NE:E has been shown to decrease during prolonged steady state exercise, (Hartley, *et al.*, 1972; Galbo, *et al.*, 1975; Christensen, *et al.*, 1979), particularly in response to a carbohydrate poor diet to deplete muscle glycogen (Galbo, *et al.*, 1979). However, Jansson, *et al.*, (1982) reported an increase in the NE:E ratio during prolonged exercise, and a difference in the time course of the increase in NE and E in response to the exercise stimulus. In the Jansson study, plasma E concentration increased only during the first 5 minutes of exercise at 65%  $\dot{V}O_{2max}$ , whereas a continuous progressive increase in plasma NE was observed throughout 25 minutes of the constant work rate protocol. Schwarz and Kindermann (1990) also reported an increase in the NE:E ratio in response to an exhaustive incremental exercise test in which NE increased 7-fold, and E increased 5-fold, from their respective resting concentration.

## CATECHOLAMINES AND VENTILATION

Circulating catecholamines have been linked to the mediation of exercise hyperpnea. The earliest report of the action of catecholamines on respiration was by Oliver and Schafer (1895) who observed a depression in respiration in dogs and rabbits following intravenous injection of extracts of the suprarenal capsule (Oliver and Schafer, 1895, cited in Joels and White, 1968). The reduction in ventilation produced by a large dose of catecholamines was later considered to be a reflex response to a concomitant rise in arterial pressure, mediated by impulses in the aortic and carotid baroreceptors through the vagus and carotid sinus nerves (Wright, 1930; Langdren and Neil, 1951). Similar experiments using smaller, more controlled doses of catecholamines produced an increase in ventilation in

anaesthetized cats and dogs (Nice,*et al.*, 1914). In man, intravenous infusion of small doses of either epinephrine or norepinephrine produces a transient stimulation of ventilation (Whelan and Young, 1953; Cunningham,*et al.*, 1963; Joels and White, 1968).

The increase in ventilation observed in response to small doses of catecholamines has been attributed to a central mechanism (Nice,*et al.*, 1914), to a general stimulation of metabolism (Boothby and Sandiford, 1923), or mediated by arterial chemoreceptors (Joels and White, 1968). Part of the stimulation of the peripheral chemoreceptors by catecholamines may also result from vasoconstriction of the extensive vasculature of the chemoreceptors, inducing local hypoxia (Llados and Zapata, 1978). An earlier study, which reported that the lower threshold of the hypoxic ventilatory response was not changed by noradrenaline infusion in humans, suggests that the circulation to the peripheral chemoreceptors was not affected (Cunningham,*et al.*, 1963). Joels and White (1968) showed that intravenous infusions of epinephrine and norepinephrine increased the minute ventilation in anaesthetized cats breathing room air, and also increased the respiratory responses of anesthetized animals to hypoxia and hypercapnia. The increase in ventilation in the latter study was accompanied by an increase in carotid body chemoreceptor discharge, but was abolished by bilateral sectioning of the carotid sinus nerves.

## TEMPERATURE

Elevated body temperature may also contribute as a ventilatory stimulant both at rest and during exercise. Hales *et al.*, (1970) demonstrated in cross-perfused dogs that a comparable change in rectal temperature similar to that expected during severe exercise (i.e. 2-3 °C), but produced solely by external heating, was associated with a large increase in  $\dot{V}_E$  from 6 to 52 l·min<sup>-1</sup>. In humans, at a constant  $\dot{V}_E$ , increased core temperature ( $T_C$ ) produced either by exercise or passive heating alters breathing pattern by decreasing tidal volume ( $V_T$ ), and increasing frequency of breathing ( $f$ ), (Martin,*et al.*, 1979). This decrease in  $V_T$  is associated with a shortened inspiratory time ( $T_I$ ), although the drive

( $V_T/T_I$ ) and timing ( $T_I/T_{TOT}$ ) components of ventilation are unchanged by elevation of body temperature.

### TEMPERATURE-MEDIATED STIMULATION OF VENTILATION

Temperature may mediate its effect on ventilation by one of several mechanisms. An early investigation of the involvement of the carotid chemoreceptors in respiratory control reported an increased sensitivity of the carotid chemoreceptors at elevated temperatures, in dogs with vascularly isolated carotid bodies. Minute ventilation was increased when carotid body temperature was increased above normal body temperature by a warm perfusate, and conversely it decreased when carotid body temperature was below normal (Bernthal and Weeks, 1939). Eyzaguirre and Lewin (1961) provided further evidence of peripheral chemoreceptor sensitivity. They observed that isolated carotid bodies *in vitro* increased the frequency of their discharge in response to an increase in the temperature of the bathing medium above 37°C. However, a slowing of the response was observed, within 3-5 minutes at temperatures greater than 37°C (Eyzaguirre and Lewin, 1961), suggesting that the increased sensitivity of the peripheral chemoreceptors to elevated temperature shows adaptation. Later, McQueen and Eyzaguirre (1974) suggested that the temperature effect on the sensitivity of peripheral chemoreceptors could be mediated indirectly by a temperature-dependant change in enzyme activity, altered blood gas tensions and pH, blood viscosity, and blood flow through the region. Cunningham and O'Riordan (1957) also observed that passive elevation of body temperature by 1°C in humans increases the respiratory sensitivity to CO<sub>2</sub>.

The increase in muscle temperature produced by exercise may also stimulate thermoreceptors within muscle leading to an increased  $\dot{V}_E$ , although direct microwave heating of muscle in the absence of exercise did not stimulate ventilation (Morgan *et al.*, 1955). Bligh (1966) proposed that an increase in blood temperature stimulates  $\dot{V}_E$  by excitation of both central (hypothalamic) and peripheral thermoreceptors. Kniffki *et al.*,

(1981) observed that approximately 50% of the group III and group IV afferent neurons innervating skeletal muscle alter their discharge frequency in response to thermal stimuli within the physiological range. Increased body temperature also affects arterial blood gas tension and pH, by decreasing pH and increasing PCO<sub>2</sub> and PO<sub>2</sub> (Ashwood *et al.*, 1983), which in turn may increase the ventilatory stimulus. Cooper and Veale (1986) proposed that raised body temperature could mediate the increase in  $\dot{V}_E$  by a direct action on a central respiratory pacemaking system.

Although increased core temperature may contribute to ventilatory stimulation during prolonged exercise, body temperature does not change quickly enough to be related to the rapid change in  $\dot{V}_E$  observed at the onset or termination of exercise (Lambertsen, 1980), or at the ventilatory inflection points observed during ramp incremental exercise to exhaustion.

## POTASSIUM

Several studies on the control of breathing during exercise have suggested that arterial [K<sup>+</sup>] plays an important role in exercise hyperpnea (Kilburn, 1966; Band *et al.*, 1982; Linton *et al.*, 1984; Sneyd and Wolfe, 1988; Busse *et al.*, 1989; Paterson *et al.*, 1989a; Newstad *et al.*, 1990; Paterson *et al.*, 1990; Yoshida *et al.*, 1990). Busse *et al.*, (1989) reported a strong correlation ( $r=0.90$ ,  $p<0.001$ ) between plasma potassium concentration and ventilation during prolonged exercise in humans. Ramp incremental exercise to exhaustion (Yoshida *et al.*, 1990), and maximal exercise on a cycle ergometer (Patterson *et al.*, 1989a), also produced an elevation in human arterial [K<sup>+</sup>] which was closely related to a change in ventilation. Additional evidence to support an important role of [K<sup>+</sup>] in the stimulation of ventilation during exercise was provided by Patterson *et al.*, (1990), who observed a close temporal relationship between the increase in arterial [K<sup>+</sup>] and  $\dot{V}_E$  during incremental exercise in subjects with McArdle's syndrome who did not become acidotic during exercise. In normal subjects, arterial [K<sup>+</sup>] was increased by 2 mM

during maximal incremental exercise; subjects with McArdle's syndrome responded to incremental exercise with a similar increase in arterial  $[K^+]$ , although their work capacity was significantly less than that of a normal subject.

#### **SOURCE OF PLASMA POTASSIUM DURING EXERCISE**

During exercise, the principle mechanism responsible for the rise in plasma  $[K^+]$  is the release of  $K^+$  from contracting muscle (Kilburn, 1966; Van Beaumont *et al.*, 1981; Sahlin and Broberg, 1989), a decrease in plasma volume (Edwards *et al.*, 1983; Harrison, 1986), and possible release of  $K^+$  from erythrocytes (Reinhart *et al.*, 1983; Hespel *et al.*, 1986). During muscle activity, potassium leaks from skeletal muscle cells due to depolarization of the membrane, and is transported back to the cell by Na-K ATPase, or the Na-K pump (Laurell and Pernow, 1966; Kilburn, 1966). The leak of potassium from active skeletal muscle to interstitial fluid exceeds the amount that can be taken up by the Na-K pump between contractions (Clausen *et al.*, 1987; Everts *et al.*, 1988). The progressive increase in plasma  $[K^+]$  with exercise has been interpreted to reflect a continuing release of  $[K^+]$  from the contracting muscles (Kilburn, 1966; Van Beaumont *et al.*, 1981; Sahlin and Broberg, 1989). This has been confirmed in humans from the reduced muscle intracellular  $[K^+]$  found at the end of exercise (Lindenger and Sjogaard, 1991). The potassium concentration in the interstitial space of skeletal muscle may increase to 8-15 mmol·l<sup>-1</sup> during muscle contraction in animals (Hnik, *et al.*, 1976) and in human subjects (Knellmer, 1961; Vyskocil *et al.*, 1983). Interstitial potassium equilibrates with plasma, causing a rise in plasma potassium during exercise.

#### **MECHANISM OF STIMULATION OF VENTILATION BY POTASSIUM**

The mechanism whereby potassium may influence ventilation remains equivocal, although several recent papers suggest that excitation of arterial chemoreceptors by  $[K^+]$  could be an important factor in the control of exercise hyperpnea. Increasing arterial  $[K^+]$

by intravenous infusion into anaesthetized animals, to a value comparable with that observed during exercise, produces an increased discharge of arterial chemoreceptors without significant change in arterial pH (Band *et al.*, 1985; Linton and Band, 1985; Band and Linton, 1986; Paterson and Nye, 1988). Band *et al.* (1985) have also demonstrated that stimulation of  $\dot{V}_E$  by hyperkalemia in the cat is abolished when both the aortic and carotid body chemoreceptor nerves are sectioned. In addition, Paterson and Nye (1988) demonstrated that the carotid chemoreceptor discharge increased more steeply at a higher arterial potassium concentration, indicating that the carotid chemoreceptors are significantly more sensitive at the higher (6.0-8.0 mM) rather than at the lower (4.0-6.0 mM) range of arterial  $[K^+]$ . Cunningham, *et al.*, (1966) also observed much earlier that the carotid bodies contribute an increasingly significant drive to ventilation during extended exercise. It has been suggested that this increase may be attributed, in part, to increased sensitivity of the carotid chemoreceptors by raised  $[K^+]$  (Paterson and Nye, 1988). Based on the Nernst equation, an increase in extracellular  $[K^+]$  would decrease the transmembrane potential of a cell. During exercise in which the  $[K^+]$  increases from 4.0 mM to 5.5 mM, a decrease in membrane potential from -95 mV to -85 mV would be expected, based on the change in  $[K^+]$  alone, which could easily account for an increase in the sensitivity of the carotid chemoreceptors (Linton, *et al.*, 1984).

One argument against a significant role for potassium in the central control of ventilation is that a reduction in arterial  $[K^+]$  by 2 mM in subjects with chronic hyperkalemia due to renal insufficiency does not affect ventilation at rest (Paterson, *et al.*, 1989b). Extrapolation of these results to normal individuals during exercise is questionable however, since (i) ventilatory measurements were made in patients at rest, and (ii) other complications as a result of renal disease, such as decreased arterial chemoreceptor sensitivity due to chronic hyperkalemia, or the concomitant metabolic acidosis and hypocapnia reported at the time of the experiment, could have obscured the ventilatory response to  $[K^+]$ .

## GLUCOSE

Although the concentration of blood glucose during exercise may have no direct affect on pulmonary ventilation, it is possible that an exercise-induced fall in blood glucose concentration may enhance catecholamine release, which would secondarily stimulate ventilation. Galbo, *et al.*, (1979) observed that the rate of decrease in blood glucose during prolonged exercise was accompanied by an increase in the plasma epinephrine and norepinephrine concentration. When a decrease in plasma glucose was avoided, by glucose infusion throughout exercise, the catecholamine response to exercise was reduced (Galbo, *et al.*, 1977a). If the plasma glucose concentration was restored to its pre-exercise concentration by late glucose infusion, plasma epinephrine concentration decreased, while norepinephrine concentration remained constant (Galbo, *et al.*, 1979).

Hypoglycemia, by reflex activation of the sympathetic nervous system, may be accompanied by an increased heart rate, weakness, and sweating. A generalized increase in circulating catecholamines would likely affect ventilation by stimulation of peripheral chemoreceptors (Cunningham, *et al.*, 1963; Joels and White, 1968; Berger and Hornbein, 1989).

## AMMONIA: A POTENTIAL VENTILATORY STIMULANT DURING EXERCISE

Ammonia has been shown to stimulate ventilation in the absence of hypercapnia both in animals and in man with intact respiratory control systems. In animals, hyperventilation has been induced experimentally by intravenous infusion (dogs) (Roberts, *et al.*, 1956) or intraperitoneal injection (rats) (Hindfeldt and Siesjo, 1971) of ammonium acetate, and by intravenous or intraventricular infusion of buffered ammonium chloride in dogs (Wichser and Kazemi, 1974).  $\dot{V}_E$  was increased both by an increase in  $V_T$  and  $f$  (Wichser and Kazemi, 1974).



In man, increased  $\dot{V}_E$  has been correlated with an elevated blood ammonia concentration in diverse clinical conditions including hepatic coma, Reye's syndrome, and in congenital or acquired defects of enzymes of the urea cycle (Wichser and Kazemi, 1974; Herrera and Kazemi, 1980). Alveolar hyperventilation and respiratory alkalosis are both present when blood ammonia is elevated in these conditions. The exact mechanism by which ammonia acts as a ventilatory stimulant is unknown, but the consensus is that it affects the central control of ventilation (Wichser and Kazemi, 1974; Dutton and Berkman, 1978; Herrera and Kazemi, 1980; Cooper and Plum, 1987).

It is surprising that there is no information in the literature concerning deviation from normal ventilation in patients with myoadenylate (AMP) deaminase deficiency (MADD), who do not produce ammonia during exercise.

#### AMMONIA PRODUCTION AND CLEARANCE DURING EXERCISE

Ammonia production by active skeletal muscle depends on exercise intensity and duration, which determine the demand for ATP formation (Babij, *et al.*, 1983; Banister, *et al.*, 1983; Buono, *et al.*, 1984; Eriksson, *et al.*, 1985; Graham, *et al.*, 1987; Katz, *et al.*, 1986). The extent of motor unit or muscle fibre recruitment (Henneman and Mendell, 1984), relative muscle fibre composition (Dudley, *et al.*, 1983), environmental conditions (Graham, *et al.*, 1987; Young, *et al.*, 1987), and the state of training of an individual (Lo and Dudley, 1987) are also contributing factors.

The reason for ammonia accumulation, and the source of ammonia production during exercise is the subject of considerable debate. In short term intensive exercise, skeletal muscle becomes a major source of ammonia production during exercise (Sahlin, *et al.*, 1978; Meyer, *et al.*, 1980; Dudley, *et al.*, 1982; Katz, *et al.*, 1986) by deamination of AMP to IMP in a cyclic process called the purine nucleotide cycle (PNC) (Lowenstein and Tornheim, 1971). Deamination of amino acids during extended work which stimulates protein uptake and amino acid catabolism in skeletal muscle is another potential contributor

to the ammonia production during exercise. Alanine, glutamine, glutamate, aspartate, and the branched chain amino acids (BCAA) leucine, isoleucine, and valine, are the main amino acids metabolized in skeletal muscle (Goldberg and Chang, 1978; Lemon and Nagle, 1981). Amino acids are also metabolized in adipose tissue, where some of the carbon fragments are also converted to triacylglycerol (Tischler and Goldberg, 1980; Newsholme and Leech, 1983). Tischler and Goldberg (1980) demonstrated that branched chain amino acids are metabolized in isolated adipose tissue, with a net release of alanine and glutamine. More recently Kowalchuk, *et al.*, (1988) showed that isolated adipocytes utilize glutamine, and produce glutamate, ammonia, lactate, and alanine. However, the rate of utilization of amino acids in adipose tissue is low in comparison with other tissues, and is unlikely to be a major contributor to ammonia production during exercise (Kowalchuk, *et al.*, 1988).

Evidence supporting the contribution of amino acid metabolism to ammonia production during exercise in humans is the marked increase in alanine release and glutamate uptake (with no change in glutamine release) by the exercising leg (Eriksson, *et al.*, 1985; Katz, *et al.*, 1986). Similarly, Rennie, *et al.*, (1981) reported that branched chain amino acids are oxidized during exercise in humans. Leucine turnover and oxidation are also enhanced by exercise (Henderson, *et al.*, 1985). The absolute rate of branched-chain amino acid catabolism in skeletal muscle is low (about  $0.01 \mu\text{mol}\cdot\text{min}^{-1}\cdot\text{g}^{-1}$ ) and it would only provide about 10% of the ATP requirement of resting muscle (about  $1-2 \mu\text{mol}\cdot\text{min}^{-1}\cdot\text{g}^{-1}$ ), although the complete oxidation of the amino acids to carbon dioxide would double this rate of ATP formation (Newsholme and Leech, 1983). However, even this seemingly small amount of amino acid catabolism could contribute significantly to ammonia production, if one assumes that about 10 kg. of skeletal muscle is active in cycle ergometry. These data are inconclusive but suggest that exercise may be associated with augmented amino acid catabolism and this could contribute to the accumulation of ammonia.

Clearance of ammonia from the circulation depends on renal and hepatic uptake and elimination, and uptake by inactive skeletal muscle. The decrease in renal blood flow which occurs during exercise (Rowell, 1983), could negatively affect renal uptake and excretion of ammonia. A reduced hepatic blood flow has also been reported to accompany exercise (Felig and Wahren, 1971; Rowell, 1983; Eriksson, *et al.*, 1985), although hepatic ammonia clearance does not decrease during exercise in the range of 30-80%  $\dot{V}O_{2max}$  (Eriksson, *et al.*, 1985).

### **GLYCOGEN DEPLETION AFFECTS AMMONIA ACCUMULATION DURING EXERCISE**

Broberg and Sahlin (1988) reported a significantly greater blood ammonia concentration during submaximal exercise to exhaustion in subjects who were glycogen depleted, despite an accompanying relatively low lactate concentration. The blood  $NH_3$  also increased faster during exercise in the glycogen-depleted subjects compared with controls. This was recently confirmed by Greenhaff, *et al.*, (1991). Muscle glycogen deficiency during exercise could result in an imbalance between utilization and resynthesis of ATP, resulting in an increased concentration of both muscle ADP and AMP, each of which are known to be potent activators of AMP deaminase (Lowenstein, 1972; Wheeler and Lowenstein, 1979). A high adenine nucleotide flux in muscle is almost certainly accompanied by an increased rate of AMP deamination to IMP and  $NH_3$ . This hypothesis is supported by recent evidence showing that exercise at 68% of  $VO_{2max}$  to exhaustion which resulted in a decrease of muscle glycogen to approximately 30% of its pre-exercise value was accompanied by a marked increase in skeletal muscle IMP content (Norman, *et al.*, 1987).

During exercise when muscle glycogen decreases to a low level, an increase in IMP concentration is observed in muscle containing predominantly type I or type II muscle fibres (Norman, *et al.*, 1988). This suggests that deamination of AMP to IMP and  $NH_3$

occurs in both fibre types despite the absence of cellular acidosis. Previously it had been reported that AMP deaminase in high-oxidative muscle was activated mainly through an increase in AMP concentration, whereas cellular acidosis was the major activator in the low-oxidative muscle (Dudley and Terjung, 1985), although these data were obtained in rat skeletal muscle. Because type I fibres have a higher oxidative capacity than type II fibres, and are predominantly recruited during exercise of submaximal intensity, it was suggested by Graham, *et al.*, (1987) that perhaps type I fibres were the main source of  $\text{NH}_3$  during prolonged submaximal exercise, which could explain the observed dissociation between lactate and  $\text{NH}_3$  accumulation.

#### **POSTULATED SITE AND MECHANISM OF ACTION OF AMMONIA ON VENTILATION**

The site of action of ammonia as a ventilatory mediator is unknown. Although both peripheral and central mechanisms have been considered, there is little support for ammonia's involvement as a peripheral ventilatory stimulant. Most evidence points to a central action of ammonia as a ventilatory stimulant.

#### **Peripheral Action of Ammonia**

Eldridge (1972) reported that a solution of ammonia injected into the blood supply perfusing the carotid body of anaesthetized cats decreased the activity of the carotid sinus nerve. Closer examination of this study, however, revealed that the agent used was ammonium hydroxide (0.1N, pH=10.3) (Eldridge, 1972). The pH of this solution would silence the activity of carotid chemoreceptors, irrespective of any possible action of ammonia (Fitzgerald and Parks, 1971). These results provide little useful information about ammonia's effect on peripheral chemoreceptors. Another possible peripheral site of action of ammonia is within skeletal muscle. If chemoreceptors exist in peripheral muscle, then they could be stimulated by elevated ammonia concentration during muscle activity.

This is an untested hypothesis however, and if such muscle chemoreceptors exist, it is unlikely that they would respond specifically to ammonia.

## AMMONIA IN THE CENTRAL NERVOUS SYSTEM

Banister and Cameron (1990) postulated that during exercise, elevated blood ammonia and a change in the ratio of plasma amino acids may favour the movement of ammonia and some amino acid precursors of neurotransmitter synthesis across the blood brain barrier (BBB). To facilitate the understanding of the action of ammonia in the central nervous system, reference is made to diagrams in this review paper, included as Appendix V. Based on evidence of brain ammonia metabolism and its direct neurophysiological action, ammonia could interact in the central nervous system, and ultimately lead to symptoms of "central fatigue" (Appendix V, Fig. 7).

In contrast to the lack of information to support a peripheral action of ammonia, there is strong support for the hypothesis that ammonia stimulates ventilation at a central locus. The effects of ammonia on ventilation have been considered a consequence of its toxicity to the central nervous system (Wichser and Kazemi, 1974; Dutton and Berkman, 1978; Herrera and Kazemi, 1980). The increased ventilation, which results from either intravenous or intraventricular infusion of buffered ammonium, is strongly correlated with the level of ammonia in the CSF and brain tissue, but not with blood ammonia; with intraventricular infusion of buffered ammonium chloride, there is no elevation of blood ammonia, but  $\dot{V}_E$  is significantly increased (Wichser and Kazemi, 1974).

In order for peripherally-produced ammonia to act centrally, it must, of course, cross the blood brain barrier (BBB). It is now acknowledged that ammonia has access to the brain from the blood both as free base  $\text{NH}_3$  and as the  $\text{NH}_4^+$  ion (Dawson, 1978; Raichle and Larson, 1981; Cooper and Plum, 1987) and is directly dependent on blood pH (Stabenau, *et al.*, 1959; Waelsch, *et al.*, 1964). When ammonia is presented to brain tissue in a large single dose, at a rapid rate, or following an already established elevated

condition, existing endogenous detoxification mechanisms are unable to contain the increased ammonia load and the brain ammonia concentration rises rapidly (Gjedde, *et al.*, 1978 ; Hindfeldt, 1973).

Ammonia is an integral component of endogenous brain metabolism. Under resting conditions the ammonia content of the brain is maintained at a relatively low concentration (Appendix V, Table 1). Ammonia becomes incorporated into the glutamate-glutamine system in the brain (Benjamin and Quastel, 1975; Benjamin, 1983). Following continuous common carotid infusion of nitrogen label from [<sup>15</sup>N] ammonia, the label rapidly appears principally in the amino group of glutamate and in both glutamine nitrogens (Stein, *et al.*, 1976) (Appendix V, Fig. 4). This suggests that ammonia-nitrogen incorporation is both by transamination of  $\alpha$ -ketoglutarate and by further amidation of glutamate to glutamine. In associated reactions, glutamate may also undergo oxidative decarboxylation by glutamate decarboxylase (GAD) to form GABA (Hertz, 1979; Hertz *et al.*, 1983; Rothstein and Tabakoff, 1985) (Appendix V, Fig.7). Glutamate and GABA, respectively, have defined excitatory and inhibitory actions as neurotransmitters while glutamine has no known neurotransmitter action (Eldridge and Millhorn, 1981; Toleikis *et al.*, 1979; Chiang *et al.*, 1986).

Regional differences in the capacity for ammonia removal (buffering) have been described for brain tissue. Butterworth *et al.*, (1988) suggested that the cerebral cortex (CC) has a limited ability to remove blood-borne ammonia by the formation glutamine, compared with the brainstem. This is due to a moderate decrease of glutamine synthetase (GS) activity in the CC accompanying hyperammonemia. Thus, a regionally specific change in ammonia concentration may occur which is disruptive to local neural activity.

It is well documented that ammonia is directly neuroactive (Lux, 1971; Lux, 1974; Plum *et al.*, 1974; Raabe and Gumnit, 1975; Iles and Jack, 1981). Lux (1970, 1971, 1974) concluded that ammonia's reduction of postsynaptic inhibition at the motoneuron is predominantly mediated by an increase in the permeability of the cell to chloride ions. Iles

and Jack (1981) observed a small ( $\approx 5$  mV), but maintained, depolarization of motoneurons following administration of ammonia to anaesthetized cats. They suggested that the nerve cells may be directly permeable to ammonia through channels permeable to potassium. An increase in extracellular ammonia would thus depolarize the cell directly, and also lead to a displacement of potassium to the extracellular space. Iles and Jack (1981) proposed that the depression of postsynaptic inhibition may be responsible for many of the clinical symptoms of ammonia toxicity in humans. Neurological symptoms ascribed to hyperammonia include abnormal locomotor behavior (Holmin and Siesjo, 1974), altered sleep patterns (Beaubernard *et al.*, 1977), modification of neuromuscular coordination (Giguere and Butterworth, 1984), and as described above, hyperventilation. Wichser and Kazemi (1974) suggested that ammonia may stimulate ventilation either by acting on neurons which exert a facilitory influence upon the respiratory centres, or it could act directly on the respiratory motoneurons.

In addition to the neurophysiological effects, ammonia causes metabolic changes in glycolysis, Krebs cycle intermediate compounds, the NADH-NAD system, and organic amine metabolism in the brain (see Cooper and Plum, 1987, for review). These changes may influence respiratory control if ammonia alters neural function either by depleting various neuronal substrates, or by leading to changes in neurotransmitter concentration, or both (Dutton and Berkman, 1978). A depletion of high energy phosphates in the midbrain has been proposed as a mechanism whereby ammonia may induce hyperventilation (Schenker *et al.*, 1967). Hyperammonemic animals have an increased brain lactate-pyruvate ratio associated with a significant alteration in intracellular pH; the NADH/NAD ratio is also increased; both glucose and glycogen concentrations are decreased, and postulated to decrease ATP content, thus affecting cerebral energy supply. These effects are reported to be localized preferentially in the base of the brain (Herrera and Kazemi, 1980). If depletion of high energy phosphates occurs in the basilar section, it could explain

some of the neurological symptoms and signs, in addition to the hyperventilation associated with hyperammonemia.

Recently, short-lived isotopes ( $^{11}\text{C}$ ,  $t_{1/2} = 20$  min;  $^{13}\text{N}$ ,  $t_{1/2} = 9.9$  min) have been used to study the central chemical drive to ventilation (Kazemi, 1987). Ammonia taken up by the brain following intra-arterial injection of  $^{13}\text{N}$ -ammonia appears as glutamine, then is converted into the neurotransmitters GABA and glutamate. It was concluded that ventilatory drive is dependent on electrolyte and acid-base status of brain ECF, the interaction between  $\text{H}^+$  metabolism and  $\text{CO}_2$  fixation, and metabolism of the amino acid neurotransmitters GABA and glutamate (Weyne *et al.*, 1978; Kazemi and Johnson, 1986; Kazemi, 1987).

$^{11}\text{C}$ -labelled  $\text{HCO}_3^-$  has also been used to assess  $\text{CO}_2$  fixation in the medulla and cerebral cortex in order to compare this to changes in the amino acid concentration found at these sites during hypercapnia (Hoop *et al.*, 1985). The importance of this, is that  $\text{CO}_2$  fixation in the brain promotes entry of  $\text{CO}_2$  into the Krebs cycle through oxaloacetate, and alters the equilibrium of ammonia-related amino acids at the  $\alpha$ -ketoglutarate-glutamate level (Weyne *et al.*, 1978) (Appendix V, Fig. 7).  $\text{CO}_2$  fixation rate varies in different regions of the brain. The rate of  $\text{H}^{11}\text{CO}_3^-$  ( $\text{CO}_2$ ) fixation in the medulla is correlated positively with a concomitant increase in medullary glutamine and GABA, at a site where respiratory centres reside (Hoop *et al.*, 1985).

Confirmation of whether there is an elevation of ammonia concentration in the brain during exercise awaits development of adequate experimental techniques to determine brain ammonia flux during exercise in humans.

## **RATING OF PERCEIVED EXERTION DURING EXERCISE**

The subjective measurement of rating of perceived exertion (RPE), is an accepted and simple method of determining a subject's impression of exercise intensity (Borg, 1982). The Borg scale of perceived exertion has previously been shown to correlate



between 0.80 and 0.90 with heart rate, oxygen uptake, and lactate accumulation (Borg, 1982), and is extensively used in exercise testing (ACSM, 1990), exercise prescription (Pollock *et al.*, 1986), and in the quantification of energy expenditure (Cardio Stress Inc, 1988).

The relationship between RPE and pulmonary ventilation is also of interest. Martin *et al.* (1981) suggested that if the sensed level of breathing is an important part of the overall perception of exertion during prolonged exercise, or if significant ventilatory muscle fatigue occurs during heavy exercise, then a rising  $\dot{V}_E$  could reduce exercise tolerance. Previous evidence suggests that ventilatory function and/or discomfort contributes to perceived exertion, although the exact aspect of ventilation that is sensed is unclear (Noble *et al.*, 1973; Robertson, 1982; Demello *et al.*, 1987).

#### THE GLYCOGEN DEPLETION MODEL

Exercise in a glycogen depleted state is an appropriate model to investigate the contribution of humoral factors to exercise ventilation for several reasons. Extensive muscle biopsy work has documented the effect of exercise and diet regimens on muscle glycogen content (Bergstrom, *et al.*, 1967; Gollnick, *et al.*, 1973; Gollnick, *et al.*, 1974; Heigenhauser, *et al.*, 1983; Vollestad and Blom, 1985). A reduction in arterial pH, frequently interpreted as a primary cause of exercise hyperpnea, is attenuated at a comparable exercise-induced ventilation in glycogen depleted exercise. Thus, one or several other humoral factors must prevail as the primary ventilatory stimulant during exercise in the glycogen depleted state.

The glycogen depletion model used to examine the relationship between humoral changes and ventilation in this study is similar to that in the studies of Heigenhauser, *et al.*, (1983) and Broberg and Sahlin (1988). The former study showed that during exercise, ventilation was greater at an equivalent work rate when a subject was glycogen depleted

compared with a normal nutritional state. The authors were unable to determine the cause of the higher  $\dot{V}_E$  in the glycogen depleted trial, but they postulated that in humans, an increased "neurogenic" drive could be an important factor in the observed increase in  $\dot{V}_E$  during glycogen depleted exercise. In the study by Broberg and Sahlin (1988), it was reported that prolonged exercise to exhaustion in glycogen depleted humans resulted in a significantly higher blood ammonia concentration compared with control exercise. The ventilatory response to exercise was not reported in the latter study, however.

An elevated blood ammonia concentration has previously been identified as a ventilatory stimulant in animal studies (Roberts, *et al.*, 1956; Wichser and Kazemi, 1974; Dutton and Berkman, 1978; Herrera and Kazemi, 1980; Cooper and Plum, 1987). The question which evolved from this is whether an exercise-induced elevation in blood ammonia concentration, which is augmented during exercise in glycogen depleted subjects (Broberg and Sahlin, 1988; Greenhaff, *et al.*, 1991), is a mediator of exercise hyperpnea.

## PURPOSE OF THE CURRENT RESEARCH

The objective of this study was to investigate the relationship between the ventilatory response to exercise, and the exercise-induced production of blood ammonia and other humoral mediators of ventilation. The ventilatory response to exercise was investigated by analysis of the breathing pattern.

To achieve this objective, subjects exercised under normal and glycogen depleted conditions to produce a different ventilatory response ( $\dot{V}_E$ ) to a fixed exercise stimulus. In one condition (glycogen depletion), the effect of other known factors controlling ventilation and potentially obscuring any role of ammonia, specifically the influence of pH and  $P_aCO_2$ , are reduced or eliminated.

The following questions are addressed in the thesis.

1. What is the relationship between the increase in blood ammonia during exercise and the ventilatory response to exercise?
2. Does glycogen depletion affect the relationship between blood ammonia and exercise hyperpnea?
3. What possible mechanism(s) account for the augmented ventilation observed during glycogen depleted exercise?

## HYPOTHESES

The specific hypotheses of the study are:

1. Ammonia is a significant stimulant to ventilation during exercise.
2. The time course of developing hyperammonemia is more closely related to the ventilatory response to exercise than other potential mediators of ventilation (i.e. plasma potassium ion, pH, temperature, and plasma catecholamines).
3. The breathing pattern during exercise, as measured by changes in  $V_t, f$ , and the drive ( $V_T/T_I$ ) and timing ( $T_I/T_{TOT}$ ) components of the ventilatory cycle are related to the change in blood ammonia concentration.

## **METHODOLOGY**

### **SUBJECT SELECTION**

Five healthy male subjects 20 to 29 years of age who were physically active and capable of undertaking the specified exercise regimens described were subjects in each experiment. Four of the five subjects participated in both studies, and one additional subject had to be recruited. Informed consent was obtained from each individual after the nature and any known hazards of the study had been explained. All subjects underwent a thorough medical examination prior to their participation in the experiments which were approved by the Simon Fraser University Ethics Committee.

### **PRELIMINARY TESTS**

Anthropometric measurements (height; weight; six skinfolds measured at the triceps, subscapular, suprailiac, abdominal, front thigh, and calf sites) were obtained from each subject to determine their physical characteristics (Ross and Marfell-Jones, 1991). Body surface area (BSA), determined by the formula of DuBois and DuBois (1916), was calculated as:

$$BSA = \text{Weight}^{.425} \cdot \text{Height}^{.725} \cdot 71.84$$

Pulmonary function in each individual was measured using a Vacumed spirometer and Vacumetrics UCI-500 Spirometry software (Version 1.4) (Vacumed, Ventura California) prior to and following ramp incremental exercise to exhaustion. Forced vital capacity (FVC), forced expired volume in one second ( $FEV_1$ ), and maximum voluntary ventilation (MVV) using a 15 second procedure, were recorded.

### **GENERAL INSTRUCTIONS**

A subject was asked not to exercise for 48 hours prior to an experiment other than as required in the study. Each exercise trial was conducted in a temperature ( $21^\circ\text{C}$ ) and humidity (55% RH) controlled environment at the same time of day to avoid any diurnal

influence on physiological variables being measured. To ensure that a subject was reasonably well recovered from strenuous exercise, he was required to remain under observation for a minimum of 30 minutes following each exercise protocol.

#### **PRELIMINARY DETERMINATION OF PEAK EXERCISE CAPACITY**

Subjects were familiarized with the testing environment and exercise protocols prior to the collection of experimental data. Following familiarization, each subject completed a ramp incremental exercise test to volitional exhaustion, in order to establish their peak cycle exercise capacity. Resting ventilation was measured for 10 minutes prior to the onset of exercise. A subject began the exercise by pedalling at 80 rpm at zero watts (unloaded pedalling) on an electrically braked cycle ergometer (LODE, HL-600-R, Groningen, Holland) for 4 minutes. An tachometer (rpm) was visible to the subject. The work rate was then increased by 30 watts per minute until he could no longer maintain a pedalling frequency within  $\pm 10$  rpm of the required rate. During a 10 minute recovery period which immediately followed the exhaustive exercise, he continued to pedal at 80 rpm. From this exercise test, maximum aerobic power or  $\dot{V}O_{2\text{ max}}$  and maximum work rate ( $WR_{\text{max}}$ ) were determined. In the principal experiments of this thesis, the maximum work rate obtained during the preliminary ramp protocol to exhaustion was used to determine the individual work rate for each exercise protocol.

#### **EXPERIMENTAL DESIGN**

Following the completion of preliminary tests, each subject participated in two experimental exercise studies, one in which each subject completed ramp incremental exercise to exhaustion, and the second which involved prolonged steady state exercise. In each study, a subject exercised first in a normal, and secondly in a glycogen depleted state on separate occasions. These have been designated in the text as:

**(i) EFFECT OF RAMP INCREMENTAL EXERCISE ON VENTILATION**

**GN:** a control, normal glycogen condition

**GD<sub>ACUTE</sub>:** an acute glycogen depleted condition

**GD<sub>CHRONIC</sub>:** a chronic glycogen depleted condition

**(ii) EFFECT OF PROLONGED STEADY STATE EXERCISE ON VENTILATION**

**GN:** a control, normal glycogen condition

**GD<sub>ACUTE</sub>:** an acute glycogen depleted condition

**RAMP INCREMENTAL EXERCISE**

In this test protocol, each subject completed a ramp incremental exercise protocol as described above with minor modifications. The increment rate of the ramp protocol selected for each subject was dependent on the individual peak cycle exercise power demonstrated in the preliminary test (Results: Table 3). The intent was to ensure that the length of the exercise test was approximately equal for each subject. To achieve this, three subjects whose individual  $WR_{max}$  was close to 300 watts completed a ramp protocol with a ramp slope of  $20 \text{ W}\cdot\text{min}^{-1}$ , and two subjects whose  $WR_{max}$  was closer to 400 watts, cycled at  $30 \text{ W}\cdot\text{min}^{-1}$ .

**PROLONGED STEADY STATE EXERCISE**

In this test protocol, the individual's constant work rate was set at 50% of the  $WR_{max}$  determined in the preliminary incremental test to exhaustion. Following a 10 min period when resting ventilation was measured, each subject exercised on a cycle ergometer for 90 minutes at a work rate equal to 50% of  $WR_{max}$  or until he was unable to maintain a pedalling frequency of  $80 \text{ rpm} \pm 10 \text{ rpm}$ . This exercise session served two purposes, first to measure pulmonary ventilation and respiratory gas exchange in the GN condition, and secondly as preliminary exercise establishing glycogen depletion protocol prior to exercise in the **GD<sub>ACUTE</sub>** condition. The subject was allowed to rest for 15 minutes then

continued with the regimen described below in the section entitled Exercise to Achieve Glycogen Depletion.

### EXERCISE TO ACHIEVE GLYCOGEN DEPLETION

The control exercise condition, **GN**, was defined as the state of resting muscle glycogen in a subject following a normal, mixed diet without prior exercise within the previous 48 hours. When the intent was to deplete the muscle glycogen stores of a subject, he first completed 90 minutes of steady state exercise on a cycle ergometer at a work rate of 50% of his peak work rate determined in a preliminary exercise protocol. Following the 90 minute session a subject was allowed to rest for 15 minutes and then completed repeated intervals of supramaximal exercise in order to deplete the working muscle of glycogen further. Each interval consisted of 1 minute of cycle exercise at a work rate equal to 120% of that inducing  $\dot{V}O_{2\max}$  in the ramp exercise protocol, interspersed with 3 minutes of rest, until a full minute of exercise could not be completed. A mean number of 5.2 intervals with an average total time of 298 seconds was completed by the group. This glycogen depletion protocol was followed by 90 minutes of rest during which water, *ad libitum*, but no caloric intake was allowed. On most occasions a subject rested supine in the the laboratory. After the rest period, **GD<sub>ACUTE</sub>** exercise was completed. In the study on the effect of ramp incremental exercise on ventilation, exercise was also completed in a second glycogen depleted state, **GD<sub>CHRONIC</sub>**. In order to prepare for the **GD<sub>CHRONIC</sub>** state, following the **GD<sub>ACUTE</sub>** protocol a subject was instructed to eat only a low carbohydrate diet in the interval between the **GD<sub>ACUTE</sub>** and **GD<sub>CHRONIC</sub>** tests. Twenty four hours later, the subject returned to the laboratory to complete the **GD<sub>CHRONIC</sub>** exercise. A similar exercise protocol to reduce muscle glycogen has previously been validated by direct analysis of glycogen in biopsied muscle (Gollnick, *et al.*, 1974; Heigenhauser, *et al.*, 1983; Costill, 1988).

The initial rationale for duplicating exercise trials in the  $GD_{ACUTE}$  and  $GD_{CHRONIC}$  condition was that in the  $GD_{ACUTE}$  condition, the prior exercise needed to accomplish the depletion of muscle glycogen stores may have had a residual effect on the ventilatory response to exercise. Retesting the subject the following day in the  $GD_{CHRONIC}$  state ensured that the observed ventilatory response to exercise was due to glycogen depletion, not to a residual effect of prior exercise. Following the study on the effect of ramp incremental exercise on ventilation, it was determined that there was no significant difference between the  $GD_{ACUTE}$  and  $GD_{CHRONIC}$  condition in the ventilatory response to exercise. Thus in the study on the effect of prolonged exercise on ventilation, only GN and  $GD_{ACUTE}$  exercise was completed.

#### DIETARY INSTRUCTIONS

For GN experiments, each subject was instructed to eat a normal diet (~55% carbohydrate, 30% fat, and 15% protein), with no alcohol consumption and an emphasis on adequate hydration. In order to maintain glycogen depletion following glycogen depletion exercise, a low carbohydrate diet (~10% carbohydrate, 35% protein, and 55% fat) was maintained by a subject for the 24 hour period until the subsequent experiment. Each subject submitted a detailed diet recall sheet in each experiment. Their nutritional intake was analyzed using DIET MAC<sup>®</sup> software.

#### DATA ACQUISITION

Exercise was performed on an electrically braked cycle ergometer (LODE, HL-600-R, Groningen, Holland). Expired gas sampled at the mouthpiece was analyzed breath-by-breath for oxygen (Applied Electrochemistry, model S-3A) and carbon dioxide content (Applied Electrochemistry, model CD-3A). Inspired and expired ventilation were each measured with an Alpha Technologies Ventilation Module (model VMN110). Heart rate was continuously monitored from a wireless sensor-transmitter (Sport Tester<sup>®</sup> model



45900, Polar Electro OY, Finland). Internal body temperature ( $T_c$ ) was monitored with a rectal thermistor probe (Yellow Spring Instruments, Ohio) inserted 10 cm past the anal sphincter, connected to a Cole Parmer Thermistor Thermometer.

Electrical signals from the ventilation modules and gas analysers necessary for the analysis of ventilation and gas exchange underwent analog to digital conversion (National Instruments A/D conversion board NB-MIO-16). A software program in National Instrument LabView<sup>®</sup> (Version 2.1.1) was used to align these signals in time, correct for breath-by-breath variation in alveolar ventilation and gas exchange, and correct for the specific response time of each gas analyser (Walsh, *et al.*, 1989). Outlying data points of the ventilatory signal were eliminated from each data set before analysis. Criteria for discarding data were (i) an inspired time of less than 200 msec which indicated that the subject had swallowed, causing the expiration valve to close, and (ii) other data points which were estimated to be greater than 3 standard deviations away from the mean value.

Breath-by-breath ventilation ( $\dot{V}_E$ , BTPS),  $O_2$  uptake ( $\dot{V}O_2$ , STPD),  $CO_2$  output ( $\dot{V}CO_2$ , STPD), ventilatory equivalents for  $CO_2$  and  $O_2$  ( $\dot{V}_E/\dot{V}CO_2$ ,  $\dot{V}_E/\dot{V}O_2$ ), respiratory exchange ratio (R), tidal volume ( $V_T$ , BTPS), frequency ( $f$ ), end-tidal  $PCO_2$ , and  $PO_2$  ( $P_{ET}CO_2$ ,  $P_{ET}O_2$ ) were determined. Expired time ( $T_E$ ), and time of one complete cycle ( $T_{TOT}$ ), were measured directly, and inspired time ( $T_I$ ) was calculated as the difference between them. Drive ( $V_T/T_I$ ) and timing ( $T_I/T_{TOT}$ ) components of ventilation were also calculated (Milic-Emili, *et al.*, 1981).

Wherever cardiorespiratory variables were compared with blood variables, a data point for the former was derived by averaging breath-by-breath data for a 20 second period corresponding as closely as possible to timed blood collection.

Throughout each exercise protocol, each subject rated perceived exertion (RPE) from a Borg Scale, which ranged in estimation of the severity of exercise from 1 (very, very light) to 10 (very, very heavy work), respectively (Borg, 1982).

## BLOOD SAMPLING

Blood was sampled at rest, during unloaded pedalling, and at regular intervals throughout exercise for the analysis of ammonia, lactate, potassium, pH, catecholamines, glucose, hematocrit, and hemoglobin. In RAMP INCREMENTAL EXERCISE, a sample for ammonia, lactate, and potassium analysis was obtained at rest, at the 4th minute of unloaded pedalling, at every 2nd minute throughout the ramp, and at the endpoint of exercise. A blood sample for pH and glucose was taken at rest, at the 4th minute of unloaded pedalling, at every 4th minute throughout the ramp, and at the endpoint of exercise. This allowed adequate time for the blood gas analyzer to process each sample as soon as it was collected. A blood sample for catecholamine, hematocrit, and hemoglobin analysis was obtained at rest, at the 4th minute of unloaded pedalling, at the 14th minute of the ramp, and at the endpoint of exercise. In PROLONGED STEADY STATE EXERCISE, samples for ammonia, lactate, and potassium analysis were obtained at rest, at the end of unloaded pedalling, every 2.5 minutes up to 50 minutes, and every 5 minutes to the endpoint of exercise. A blood sample for pH and glucose was taken at rest, at the 5th minute of unloaded pedalling, at every 5th minute up to 50 minutes, and every 10 minutes to the endpoint of exercise. A blood sample for catecholamine, hematocrit, and hemoglobin analysis was obtained at rest, at the 5th minute of unloaded pedalling, at 20 minutes, and at the endpoint of exercise.

Two sites were used to obtain blood samples during exercise. An indwelling catheter (Angio-Set, 16 Ga  $1\frac{1}{8}$  in), placed in an antecubital vein and kept patent with sterile heparinized saline, was used to draw samples for analysis of ammonia, lactate, catecholamines, potassium, hematocrit and hemoglobin. Arterialized venous blood samples for blood gas and blood glucose analysis were obtained from an indwelling catheter (Angio-Set, 22 Ga  $\frac{3}{4}$  in) in a superficial vein on the dorsum of the hand which was heated by an electrically heated glove controlled by a thermister to improve in the arterialization of the blood (Forster, *et al.*, 1972). A heparinized syringe was used to

obtain a sample for blood gas and glucose analysis. Each blood sample was drawn into a syringe through a 2-way stopcock connected to an adapter on the catheter. Upon sampling, venous blood was divided immediately and transferred to a series of tubes prepared for specific metabolites. Blood was transferred to a tube containing 45 USP Units sodium heparin for ammonia analysis, iced 0.6 N perchloric acid ( $\text{HClO}_4$ ) for lactate analysis in a 2:1 volume ratio of  $\text{HClO}_4$  to whole blood, 40  $\mu\text{l}$  7.5% EDTA( $\text{K}_3$ ) for catecholamine, hematocrit and hemoglobin analysis, or into a plain vacutainer SST<sup>®</sup> tube with gel and clot activator for potassium analysis. Samples for ammonia, lactate, and catecholamines were immediately placed on ice. Samples were then centrifuged at 2,500 rpm for 10 minutes, (Clini-Cool Refrigerated Centrifuge, Damon IEC), separated, and the supernatant frozen on dry ice within 30 min of sampling before transfer to a  $-80^\circ\text{C}$  freezer and storage until analysis. Ammonia was analyzed within 24 hours of obtaining the sample due to the labile nature of the metabolite. Lactate was analyzed within one week. Samples for potassium analysis were allowed to clot at room temperature, and centrifuged to separate the serum. Hematocrit, hemoglobin and potassium analyses were performed on the day of sampling at B.C. Biomedical Laboratories, in Burnaby, British Columbia. A few samples that showed obvious hemolysis were not analyzed.

## **BLOOD CHEMICAL ANALYSIS**

A summary of the method used in each biochemical analysis, and the coefficient of variation of the method determined in this study is shown in Appendix II. The concentration of ammonia and lactate were determined on a Beckman DU-8 spectrophotometer. Blood lactate concentration was analyzed from the perchloric acid supernatant. The reaction, catalyzed by lactate dehydrogenase in the presence of excess  $\text{NAD}^+$ , results in an increased absorbance at 340 nanometers due to NADH formation in proportion to the concentration of lactate present in the sample (Sigma, 1989). The

coefficient of variation determined for this assay was 3.1%. Plasma ammonia was determined using glutamate dehydrogenase to catalyze conversion of NADPH to NADP<sup>+</sup>. The decrease in absorbance at 340 nanometers due to the oxidation of NADPH is proportional to the ammonia concentration (DCL, 1989). For the determination of ammonia, the coefficient of variation was 4.6%.

Determination of plasma catecholamine concentration was performed by high-performance liquid chromatography (HPLC) in combination with electrochemical detection as described by (Mefford, *et al.*, 1981), with modifications described below. Detection equipment included a Hewlett Packard (HP) HPLC 1050 Series isocratic pump and autosampler in combination with a HP 1049A programmable electrochemical detector with a thin layer glassy carbon electrode vs a solid state internal AgCl electrode. The detector was set at an applied working potential of 550 mV, previously determined to be the optimum detection potential for this assay. The catecholamines were separated on a reverse phase 5 micron, 125 X 4 mm column. The mobile phase contained 0.1 M monochloroacetic acid, 0.2 mM EDTA, 0.01 M KCl and 100 mg·l<sup>-1</sup> sodium octyl sulphate. pH was adjusted to 3.0 with solid NaOH. The flow rate was 1.0 ml·min<sup>-1</sup>. The chromatogram was analyzed by computer integration of the peak areas, by a Baseline 810 Chromatography workstation (Dynamic Solutions, 1989). A 500 µl plasma sample was prepared for analysis by solid phase extraction using Supelclean LC-WCX SPE tubes (Supelco, Canada). An internal standard of 25µl 87.2nM dihydroxybenzamine (DHBA) was added to each sample during extraction for a final extraction volume of 250 µl in 0.1 M HClO<sub>4</sub>. The recovery rate of the extraction procedure for the internal standard in the plasma samples averaged 62%. Each sample was analyzed in duplicate, with an injection volume of 100 µl. On each day that assays were performed, a standard curve was prepared to evaluate the linearity of the response in the anticipated concentration range of exercise catecholamines. Standard solutions of epinephrine in the range from 0.404 to 5.05 nM representing 6.84 to 85.44 pg per injection on the column, and norepinephrine from 0.844

to 10.55 nM representing 15.46 to 193.26 pg per injection showed a linear response. The coefficient of variation for epinephrine was 11.3% (determined from standard solutions only), and 7.4% for norepinephrine (determined from duplicate analysis of blood samples). In the chromatograms of the plasma samples, interference by a large unidentified peak at the retention time of epinephrine prevented its accurate determination. The contaminating peak was present in the majority of the samples analyzed. Therefore only plasma norepinephrine concentration was reported in this study.

Whole blood glucose was determined immediately using a Lifescan One Touch<sup>®</sup> glucose analyzer. Determination of glucose in the sample depended on a glucose oxidase method specific for D-glucose (Marks and Dawson, 1965). When blood was applied to the reagent test strips, glucose oxidase triggered the oxidation of glucose in the blood, forming gluconic acid and hydrogen peroxide. Peroxidase then catalyzed the reaction of hydrogen peroxide with dyes which produced a blue colour when oxidized. The intensity of blue color formed correlated with the concentration of glucose in blood. Glucose determination using a Lifescan One Touch<sup>®</sup> glucose analyzer had a coefficient of variation determined of 5.4%. The accuracy of this method of glucose analysis was tested by analyzing duplicate blood samples (n=10) with the Lifescan One Touch<sup>®</sup> glucose analyzer and by B.C. Biomedical Laboratories, where a hexokinase and glucose-6-phosphate dehydrogenase catalyzed method of glucose determination was performed (Schmidt, 1961) on a Boehringer Mannheim-Hitachi 717 analyzer. Daily standard calibration of the instrument was made using a Boehringer Mannheim "BMC-Automated Systems" solution. A correlation coefficient of 0.87 ( $p \leq 0.001$ ) was found between the two methods in a resting blood glucose range of 3.6 to 8.4 mM. Hematocrit and hemoglobin were analyzed using a "Coulter Systems" stacker automated hematology analyzer standardized daily by Coulter "S-Cal". Hemoglobin was determined using a cyanmethaemoglobin method (Lynch, 1976), and hematocrit was determined by calculation (Coulter, 1988). Potassium was measured using an ion selective electrode on a Boehringer Mannheim-Hitachi 717 analyzer

calibrated for potassium with Boehringer Mannheim "Precical". B.C. Biomedical Laboratories reported an average daily coefficient of variation of 0.9 and 0.7 for hematocrit and hemoglobin, respectively, and a coefficient of variation of 1.5% for normal and 1.1% for high potassium ion concentrations.

Blood volume (BV), red cell volume (CV) and plasma volume (PV) were calculated from values of Hb and Hct before, during, and at the end of exercise, using a series of equations derived by Dill and Costill (1974). Subscripts B, and A, denote BEFORE dehydration and AFTER dehydration.  $BV_B$  was considered to represent 100%.

$$BV_A = BV_B(Hb_B/Hb_A)$$

$$CV_A = BV_A (Hct_A)$$

$$PV_A = BV_A - CV_A$$

$$\Delta BV, \% = 100(BV_A - BV_B)/BV_B$$

$$\Delta CV, \% = 100(CV_A - CV_B)/CV_B$$

$$\Delta PV, \% = 100(PV_A - PV_B)/PV_B$$

Using these equations, the percentage change in plasma volume in response to exercise was calculated from values for Hb and Hct before and after exercise. The observed change in plasma volume was used to correct the concentration of each metabolite to account for any plasma fluid shift with exercise (Dill and Costill, 1974).

## BLOOD GAS ANALYSIS

Arterialized venous blood was analyzed for  $PO_2$ ,  $PCO_2$ , and pH using standard microelectrodes calibrated before and after each experiment with certified standard buffers and gases (CIBA Corning Canada Inc., Blood Gas Analyzer Model 178). Each blood sample was analyzed at 37°C and was corrected to  $T_c$  of the subject using formula specific for a Corning 178 analyzer (Ashwood, *et al.*, 1983). Values obtained for  $PO_2$  (too low) and  $PCO_2$  (too high) indicated that complete arterialization of the blood did not occur. Thus, only pH was used for further analysis.

## STATISTICAL ANALYSIS

### Descriptive Data and Determination of Significance

The preliminary subject data are reported as the group mean, and the range of each variable. All other data in the text and graphs are described by descriptive statistics including the mean and standard error of the mean (SEM). Each dependent variable was analyzed by a two way analysis of variance (ANOVA) with repeated measures. Statistical difference between means was assessed by a *post hoc* Bonferroni test (SAS, 1985). Significant differences were accepted at an  $\alpha \leq 0.05$  level. In the text, data are described as significant ( $p \leq 0.05$ ) or non-significant ( $p > 0.05$ ).

### Interaction Analysis

The two independent variables analyzed were condition (Control, Acute, and Chronic) and time. Significant differences were sought according to the variation calculated for CONDITION, TIME, and their interaction (CONDITION X TIME). When the interaction was not significant, the main variable effect (CONDITION and TIME) was interpreted without further qualification. A significant interaction showed that the change in a variable throughout time was different depending on the exercise condition. Data were then analyzed by one way ANOVA for CONDITION or for TIME (SAS, 1985).

### Sample Size, and Graphical Presentation of Data

All the data were time dependent, and because each subject exercised for a different duration before exhaustion, there was an unequal sample size for the variables at certain times within the group and between conditions.

In prolonged steady state exercise, the graphic representation of the group mean data in the GN condition represent  $n=5$  subjects at all time points up to 50 minutes of exercise, and  $n=4$  for the time points from 55 to 90 minutes. In the GD<sub>ACUTE</sub> condition,

and 5 subjects were able to complete 25 minutes of exercise, and the group mean data represent an  $n=5$  up to that point. The Endpoint also represents  $n=5$  subjects in both treatments. The graphs were plotted up to 25 minutes of exercise and including the endpoint, to avoid the impression of discontinuity because of the difference in the number subjects at each data point. Also, to include all subjects in the analysis, statistical comparison of the GN and GD<sub>ACUTE</sub> data by ANOVA could only be done up to 25 minutes of exercise, and at the endpoint of exercise.

In ramp exercise, the presented group mean data represent data for  $n=5$  subjects at all time points, with one exception. At  $t=18$  minutes,  $n=4$  in the GN condition, and  $n=2$  in the GD<sub>ACUTE</sub> and GD<sub>CHRONIC</sub> conditions, respectively. The group data at Endpoint represents  $n=5$  subjects in all treatments. To include all subjects in the analysis, statistical comparison of the three exercise conditions by ANOVA excluded the 18th minute of exercise. The data at the 18th minute of exercise are included in the graphs, but in some cases give an impression of discontinuity.



## RESULTS

### PRELIMINARY TESTS

A series of preliminary tests were carried out on the subjects to determine their suitability as subjects for this study. All subjects were fit and active in recreational sports. The physical characteristics of the subjects participating in these experiments are shown in Table 1. Resting pulmonary function data of each subject, including FVC measured before and after ramp incremental exercise to exhaustion are shown in Table 2.

**Table 1 Physical characteristics of subjects.**

Subject ID #	Age (years)	Height (cm)	Weight (kg)	SOS* (mm)	BSA** (m <sup>2</sup> )
1	22	184	75	44.6	1.97
2	22	179	70	48.8	1.88
3	30	178	66	57.0	1.82
4	26	168	64	47.4	1.72
5	27	178	67	33.1	1.84
6	25	187	80	51.2	2.05
Average	25.3	179.0	70.3	47.0	1.91
Range	(22-30)	(168-187)	(64-80)	(33.1-57.0)	(1.72-2.05)

\*SOS: sum of six skinfolds (Ross and Marfell-Jones, 1991)

\*\*BSA=Weight<sup>0.425</sup> • Height<sup>0.725</sup> • 71.84 (DuBois and DuBois, 1916)

**Table 2 Resting pulmonary function measurements of subjects.**

Subject ID #	FVC (pre) litres	FVC (post) litres	FEV 1 litres	MVV litres•min <sup>-1</sup>
1	5.37	5.52	4.35	182.9
2	5.35	5.28	4.85	226.8
3	6.18	5.53	5.55	199.4
4	5.22	-	4.59	214.1
5	6.95	6.71	5.03	207.4
6	6.18	6.03	5.16	239.4
Average	5.88	5.81	4.92	211.7
Range	(5.22 - 6.95)	(5.28-6.71)	(4.35-5.55)	(182.9-239.4)

Volumes are in litres BTPS. FEVC, Forced Expired Vital Capacity; FEV 1, Forced Expired Volume in 1 sec; MVV, Maximum Voluntary Ventilation.

The maximum aerobic power or  $\dot{V}O_{2\max}$  and maximum work rate ( $WR_{\max}$ ) were determined from a ramp incremental exercise protocol at  $30\text{ W}\cdot\text{min}^{-1}$ , from unloaded (0 watt) pedalling to exhaustion. Individual and group data are shown in Table 3.

In each experiment a subject submitted a detailed diet recall sheet, and their nutritional intake was analyzed using DIET MAC<sup>®</sup> software. A summary of the dietary analysis is shown in Table 4. Subject #4 did not participate in a **GD**<sub>CHRONIC</sub> protocol, and did not submit a diet recall.

**Table 3** Physical work capacity of subjects determined on a cycle ergometer.

Subject ID #	$\dot{V}O_{2\max}$ ( $\text{l}\cdot\text{min}^{-1}$ )	$\dot{V}E_{\max}$ ( $\text{l}\cdot\text{min}^{-1}$ )	$WR_{\max}$ (watts)	$HR_{\max}$ ( $\text{b}\cdot\text{min}^{-1}$ )
1	4.96	170	413	192
2	3.63	149	311	198
3	3.81	183	298	201
4	4.32	143	300	196
5	3.68	167	324	202
6	5.26	186	404	182
Average	4.28	166	342	195
Range	(3.63-5.26)	(143-186)	(298-413)	(182-202)

**Table 4** The dietary composition of normal and ketogenic diets. Protein, carbohydrate (CHO), and fat values represent the individual and group mean percentage of the daily caloric intake. Energy intake is recorded in (MJoules).

Subject ID #	Normal Diet				Ketogenic Diet			
	Protein	CHO	Fat	Energy	Protein	CHO	Fat	Energy
1	17.5	42.5	39.9	18.01	47.1	8.2	35.7	8.58
2	15.9	48.8	35.3	17.54	44.0	7.1	48.9	7.90
3	15.0	51.9	33.2	9.21	21.5	9.1	69.3	8.82
5	12.9	52.9	34.3	15.26	23.0	11.5	65.6	6.92
6	12.2	65.9	21.9	25.32	32.7	22.4	44.9	4.30
Average	14.7	52.4	32.9	17.07	33.7	11.7	52.9	7.30
Range	(12.2-17.5)	(42.5-65.9)	(21.9-39.9)	(9.21-25.32)	(21.5-47.1)	(7.1-22.4)	(35.7-69.3)	(4.30-8.82)

## PHYSIOLOGICAL RESPONSES DURING RAMP INCREMENTAL EXERCISE:

In order to compare the effect of several factors identified as potentially contributing to the ventilatory response to dynamic exercise, 5 male subjects completed incremental ramp exercise to exhaustion in normal glycogen (GN), acute glycogen depleted (GD<sub>ACUTE</sub>), and chronic glycogen depleted (GD<sub>CHRONIC</sub>) conditions. In the ramp exercise protocol, ENDPOINT was defined as the time of exercise beyond which a subject could not maintain the required pedalling frequency.

### MAXIMUM WORK RATE

Each subject completed three incremental exercise tests to exhaustion in different states of glycogen repletion. In GN exercise, a subject achieved a significantly higher maximum work rate (WR<sub>MAX</sub>), reaching  $344 \pm 27$  watts, compared to exercise in the glycogen depleted states. In the GD<sub>ACUTE</sub> and GD<sub>CHRONIC</sub> condition, the maximum work rate achieved declined to  $321 \pm 29$  and  $319 \pm 22$  watts respectively, a difference that was not significant. The group mean decrease in exercise time was one minute, from  $18.47 \pm 0.39$  min. in the GN, to  $17.46 \pm 0.37$  min, and  $17.46 \pm 0.50$  min in the GD<sub>ACUTE</sub> and GD<sub>CHRONIC</sub> conditions, respectively.

### VENTILATION

The group mean ventilatory response to incremental exercise is shown in Fig. 1, together with other gas exchange variables. The individual ventilatory response is shown in Fig. 2. Incremental exercise produced an increase in the group mean pulmonary ventilation ( $\dot{V}_E$ ) in the control, acute and chronic glycogen depletion states from  $21.0 \pm 2.2$ ,  $22.5 \pm 1.1$ ,  $21.3 \pm 1.2$  l·min<sup>-1</sup> during unloaded pedalling to  $173.5 \pm 9.5$ ,  $172.1 \pm 14.1$ , and  $161.8 \pm 10.0$  l·min<sup>-1</sup> respectively at exhaustion.  $\dot{V}_E$  tended to be higher in the

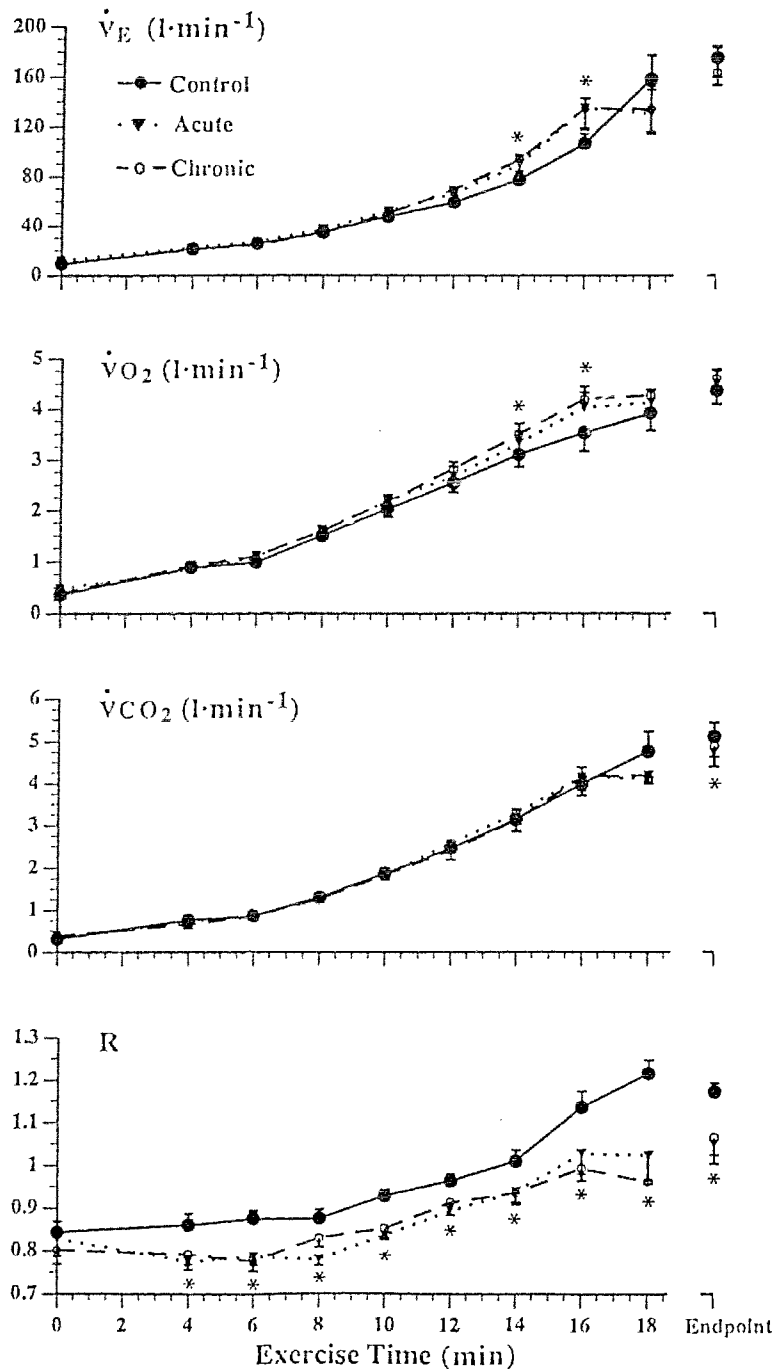
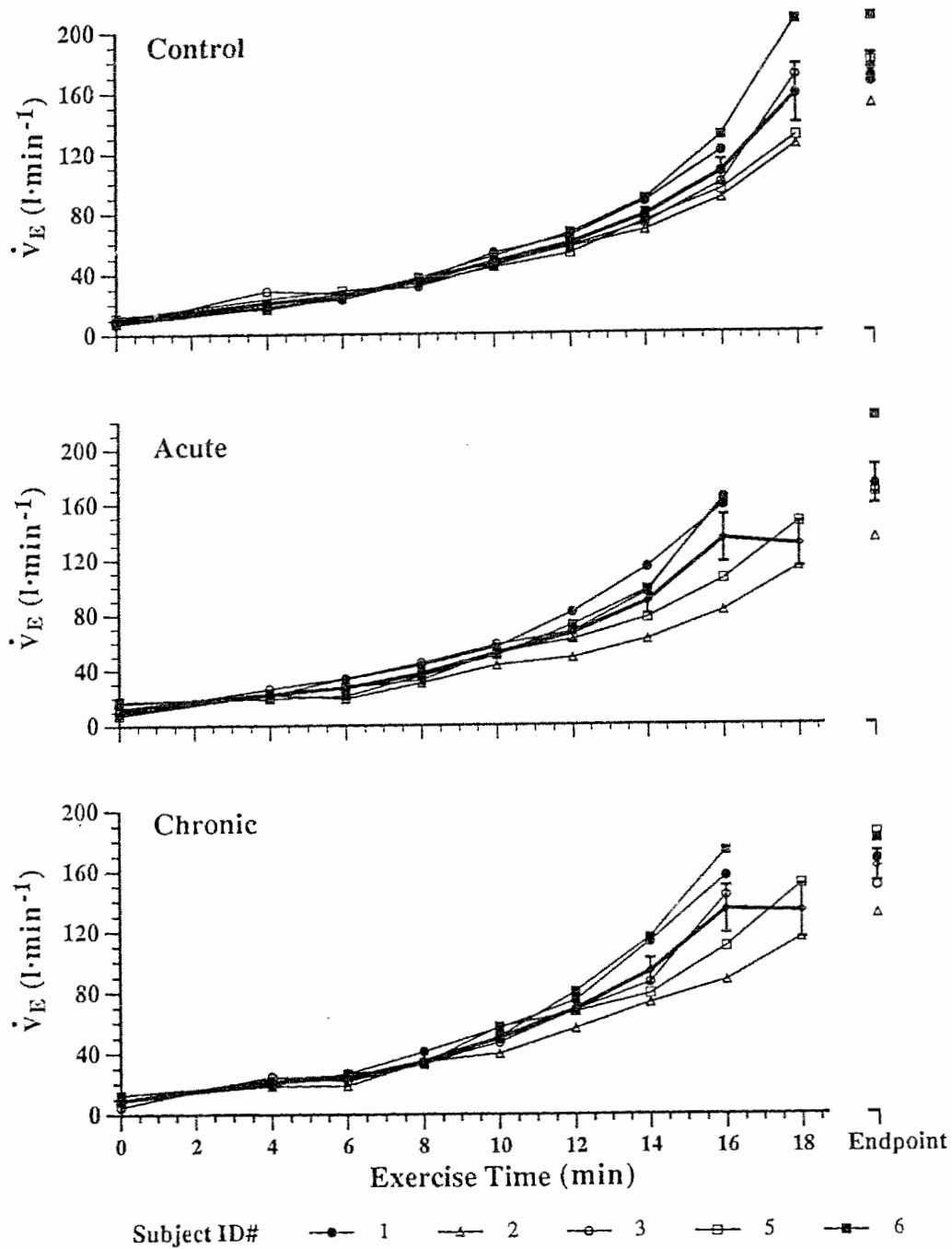


Figure 1 Ventilatory and gas exchange responses ( $\dot{V}_E$ ,  $\dot{V}_{O_2}$ ,  $\dot{V}_{CO_2}$ , and R) to ramp incremental cycle ergometer exercise to exhaustion. All values are mean  $\pm$  SEM. In each treatment, n=5 at all time points with the following exceptions. At time=18 min., n=4 in the GN treatment, and n=2 in the  $GD_{ACUTE}$  and  $GD_{CHRONIC}$  treatments. The **Endpoint** value with an n=5 in all treatments, represents the group mean value at the endpoint of exercise, irrespective of the total exercise duration of each subject. \* Significantly different from GN (control) condition at that corresponding time ( $p \leq 0.05$ ).



**Figure 2** The individual ventilatory response ( $\dot{V}_E$ ) to ramp incremental cycle ergometer exercise to exhaustion in relation to the group mean  $\dot{V}_E$ . Data for each individual subject are indicated by a subject ID number. The group mean data points are expressed as in Fig. 1.

glycogen depleted state, but the difference in  $\dot{V}_E$  between groups was significantly greater in the **GD<sub>ACUTE</sub>** and **GD<sub>CHRONIC</sub>** state relative to **GN** only between the 14th and 16th minute of exercise. The apparent, but non-representative, plateau in  $\dot{V}_E$  at the 18th minute of ramp exercise represented data from only two subjects who were able to complete this level of work while in a glycogen depleted state; because of the small n, a valid statistical comparison could not be made at this point in time, however. Even though the group mean  $WR_{MAX}$  and total exercise duration was significantly less during exercise in a glycogen depleted state compared with the control condition, maximum ventilation was  $173.5 \pm 9.5$ ,  $172.1 \pm 14.1$ , and  $161.8 \pm 10.0$  l·min<sup>-1</sup> respectively for **GN**, **GD<sub>ACUTE</sub>** and **GD<sub>CHRONIC</sub>** exercise, and not significantly different between the conditions.

#### OXYGEN CONSUMPTION

$\dot{V}O_2$  was slightly, but not significantly higher at rest in the **GD<sub>ACUTE</sub>** ( $0.48 \pm 0.02$  l·min<sup>-1</sup>) and **GD<sub>CHRONIC</sub>** ( $0.39 \pm 0.03$  l·min<sup>-1</sup>) state respectively, compared with the **GN** ( $0.36 \pm 0.04$  l·min<sup>-1</sup>) condition (Fig. 1). During unloaded pedalling, oxygen uptake no difference was observed between the three groups ( $0.88 \pm 0.06$ ,  $0.83 \pm 0.11$ , and  $0.91 \pm 0.09$  l·min<sup>-1</sup>, respectively). As the work rate increased,  $\dot{V}O_2$  increased in a linear manner in all three exercise trials.  $\dot{V}O_2$  was significantly higher in the **GD<sub>ACUTE</sub>** and **GD<sub>CHRONIC</sub>** trials compared with the **GN** trial after the 12th minute of exercise. However, at the point of exhaustion, the difference in  $\dot{V}O_2$  between conditions was no longer significant. The group mean peak oxygen consumption was slightly, but not significantly higher in the two glycogen depleted exercise conditions reaching  $4.51 \pm 0.24$  and  $4.60 \pm 0.18$  l·min<sup>-1</sup> in **GD<sub>ACUTE</sub>** and **GD<sub>CHRONIC</sub>** respectively, compared with  $4.35 \pm 0.26$  l·min<sup>-1</sup> in **GN** exercise.

## CARBON DIOXIDE PRODUCTION

In response to the ramp increment in work rate,  $\dot{V}CO_2$  also increased with increasing work rate (Fig. 1). Unlike  $\dot{V}O_2$  however,  $\dot{V}CO_2$  was not significantly different during exercise in glycogen depleted subjects with respect to control exercise. Only at the point of exhaustion was  $\dot{V}CO_2$  significantly lower in the **GD<sub>ACUTE</sub>** state compared with control, but the absolute work rate was also lower. Maximum  $\dot{V}CO_2$  was  $5.10 \pm 0.34$  in **GN** compared with  $4.72 \pm 0.31$  in **GD<sub>ACUTE</sub>** ( $p \leq 0.05$ , **GN** vs **GD<sub>ACUTE</sub>**), and  $4.89 \pm 0.26$  l·min<sup>-1</sup> during **GD<sub>CHRONIC</sub>** exercise (non-significant, **GN** vs **GD<sub>CHRONIC</sub>**).

## RESPIRATORY EXCHANGE RATIO

The effect of glycogen depletion on  $\dot{V}O_2$  and  $\dot{V}CO_2$  during incremental exercise was reflected by a significant change in the respiratory exchange ratio ( $R = \dot{V}CO_2 / \dot{V}O_2$ ).  $R$  increased throughout incremental exercise in all three exercise trials (Fig. 1).  $R$  was significantly lower during exercise in a both glycogen depleted states compared with the control condition, although no significant difference was observed for  $R$  between **GD<sub>ACUTE</sub>** and **GD<sub>CHRONIC</sub>** exercise. This difference was greatest at the endpoint, when  $R$  was  $1.17 \pm 0.02$  in the **GN** state, compared with  $1.05 \pm 0.04$ , and  $1.07 \pm 0.04$  for the **GD<sub>ACUTE</sub>** and **GD<sub>CHRONIC</sub>** conditions, respectively. Since  $\dot{V}CO_2$  was not significantly different in the three exercise conditions, the increase in  $\dot{V}O_2$  in glycogen depleted exercise was predominantly responsible for the smaller  $R$  observed.

## COMPONENTS OF VENTILATION

The ventilatory response ( $\dot{V}_E$ ) was analyzed as the product of tidal volume ( $V_T$ ) and ventilatory frequency ( $f$ ), and as the product of inspiratory drive ( $V_T/T_I$ ) and inspiratory timing ( $T_I/T_{TOT}$ ). A comparison of  $\dot{V}_E$ ,  $V_T$ ,  $f$ ,  $V_T/T_I$ , and  $T_I/T_{TOT}$  at each work rate during ramp incremental exercise in the three test conditions is shown in Fig. 3.

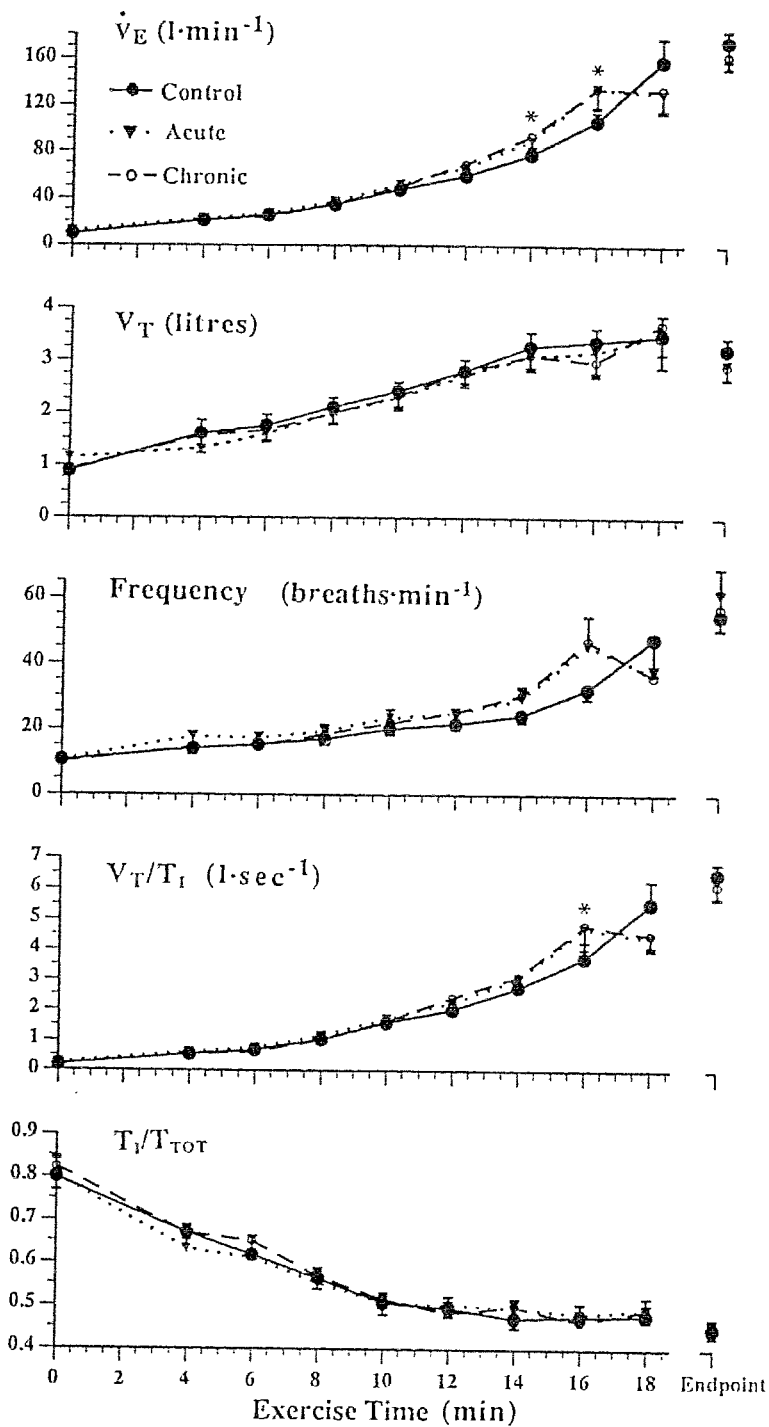
## TIDAL VOLUME

The change in tidal volume ( $V_T$ , litres) in response to ramp incremental exercise is shown in Fig.3. No significant difference was observed in tidal volume ( $V_T$ ) between any of the three conditions studied either at rest or during exercise.  $V_T$  increased with increasing work rate from a resting value of approximately one litre per minute in all three conditions to a relative plateau after the 14th minute during very intense exercise (Fig. 3). At exhaustion,  $V_T$  was  $3.22 \pm 0.23$ ,  $2.95 \pm 0.28$ , and  $2.91 \pm 0.24$  l in the respective GN,  $GD_{ACUTE}$ , and  $GD_{CHRONIC}$  conditions. This difference was not significant.

## VENTILATORY FREQUENCY

The increase in pulmonary ventilation in response to ramp exercise resulted from an increase in both tidal volume ( $V_T$ ) and breathing frequency ( $f$ ) (Fig. 3). At rest,  $f$  was the same in all three metabolic conditions (GN =  $10.4 \pm 0.7$  vs  $GD_{ACUTE}$  =  $10.6 \pm 1.1$  vs  $GD_{CHRONIC}$  =  $9.8 \pm 1.0$  breaths·min<sup>-1</sup>). During exercise,  $f$  increased with increased work intensity in all three glycogen states. There was a tendency for  $f$  to be greater during exercise in the glycogen depleted states compared to the control condition, particularly after the 12th minute of exercise when a noticeable increase in  $f$  was observed in the  $GD_{ACUTE}$  and  $GD_{CHRONIC}$  states. The difference was not statistically significant, however. At exhaustion,  $f$  was  $60.8 \pm 8.3$  breaths·min<sup>-1</sup> during  $GD_{ACUTE}$ , and  $56.8 \pm 5.7$  breaths·min<sup>-1</sup> in  $GD_{CHRONIC}$  exercise compared with  $54.6 \pm 4.2$  breaths·min<sup>-1</sup> in the GN state.





**Figure 3** Components of ventilation ( $\dot{V}_E$ ,  $V_T$ ,  $f$ ,  $V_T/T_I$ ,  $T_I/T_{TOT}$ ) in response to ramp incremental cycle ergometer exercise to exhaustion. All values are mean  $\pm$  SEM. The group mean data points are expressed as in Fig. 1. \* Significantly different from GN (control) condition at that corresponding time ( $p \leq 0.05$ ).

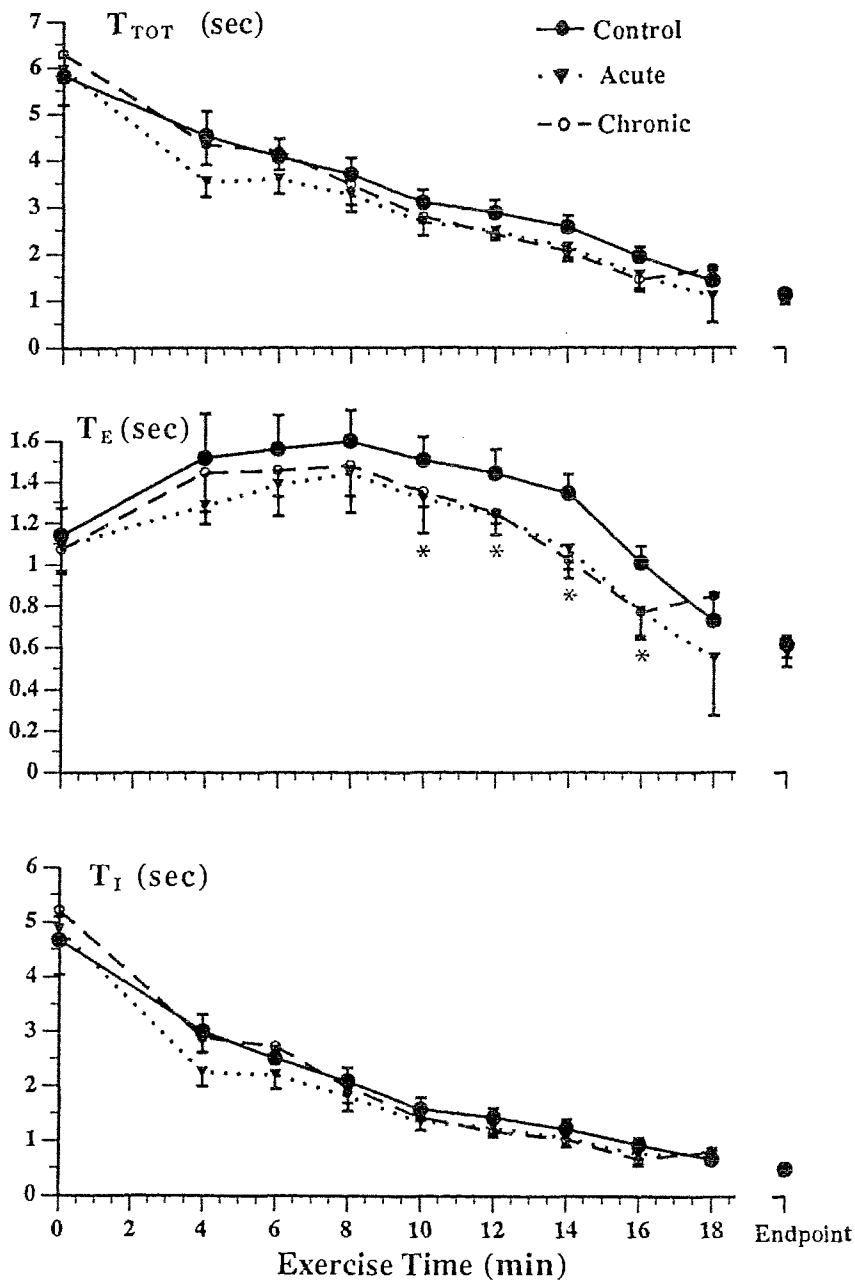
### THE DRIVE AND TIMING COMPONENTS OF VENTILATION ( $V_T/T_I$ , $T_I/T_{TOT}$ )

In the three metabolic states studied, the respiratory drive ( $V_T/T_I$ ) increased throughout incremental exercise reaching a peak value of  $6.48 \pm 0.35$ ,  $6.36 \pm 0.64$ , and  $6.11 \pm 0.43$  l·sec<sup>-1</sup> in the **GN**, **GD<sub>ACUTE</sub>** and **GD<sub>CHRONIC</sub>** conditions respectively at the exercise endpoint (Fig. 3). Except for the endpoint value,  $V_T/T_I$  was consistently larger during both glycogen depleted states than in the **GN** state, suggesting a greater ventilatory drive. The difference was only significant at the 16th minute of exercise, when  $V_T/T_I$  was significantly greater in both glycogen depleted compared with the **GN** exercise condition.

$T_I/T_{TOT}$ , a measure reflecting respiratory timing, decreased from the onset of unloaded pedalling exercise throughout the test (Fig. 3).  $T_I/T_{TOT}$  was not significantly different between exercise conditions.

### VENTILATORY TIMING: ( $T_{TOT}$ , $T_I$ , $T_E$ )

The relationship between the components of inspiratory timing ( $T_{TOT}$ ,  $T_I$ ,  $T_E$ ) at each work rate during ramp incremental exercise in the three test conditions is shown in Fig. 4. Inspiratory time ( $T_I$ ) was calculated as the difference between the total time of one respiratory cycle ( $T_{TOT}$ ) and expiratory time ( $T_E$ ) which were measured directly.  $T_I$ , which decreased from the onset of exercise, was not significantly different in the three conditions. In contrast, a small increase in  $T_E$  was observed at the lower work rates of the exercise test, followed by a significant reduction in  $T_E$  as exercise intensity increased towards exhaustion.  $T_E$  was modestly, but significantly shorter after the 10th minute of exercise in both **GD<sub>ACUTE</sub>** and **GD<sub>CHRONIC</sub>** conditions compared with the **GN** state., but the difference was not significant at the endpoint of exercise.  $T_{TOT}$ , which also decreased throughout exercise, tended to be shorter during glycogen depleted exercise, but was not significantly different from **GN** exercise.



**Figure 4** Ventilatory timing components [inspired time ( $T_I$ ), expired time ( $T_E$ ), and time of one complete cycle ( $T_{TOT}$ )] in response to ramp incremental cycle ergometer exercise to exhaustion. All values are mean  $\pm$  SEM. The group mean data points are expressed as in Fig. 1. \* Significantly different from GN (control) condition at that corresponding time ( $p \leq 0.05$ ).

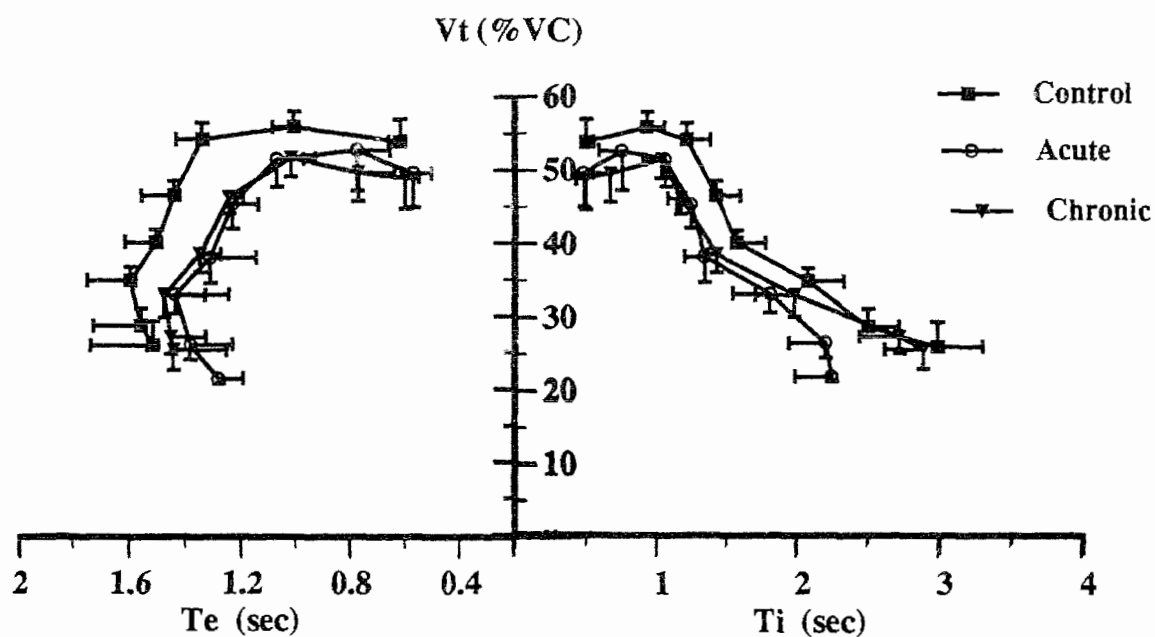
## THE RELATIONSHIP OF TIDAL VOLUME TO INSPIRATORY AND EXPIRATORY DURATION

The group mean relationship between  $V_T$  (%VC) and  $T_I$  and  $T_E$ , in response to ramp incremental exercise is shown in Fig. 5. The  $V_T$ - $T_I$ - $T_E$  diagram confirms that the increase in breathing frequency during ramp exercise (Fig. 3) was due to shortening of both  $T_I$  and  $T_E$ .

Two ranges are apparent in the relationship between  $V_T$  and  $T_I$ . At low work rates,  $T_I$  decreased linearly in relation to the increase in  $V_T$ . At high rates of work, there is an obvious breakpoint in the relationship, when  $T_I$  continued to decrease while  $V_T$  showed no further increase, or a slight decrease.

In the relationship of  $V_T$  to  $T_E$ , three ranges were discernable. In the first, at a low work rate,  $V_T$  increased rapidly without a concomitant decrease in  $T_E$ . In the second range,  $T_E$  decreased almost linearly as  $V_T$  increased with work rate. As work rate increased, there was an obvious breakpoint, as  $T_E$  decreased while  $V_T$  remained relatively unchanged.

During exercise, the relationship between  $V_T$  and  $T_I$ , and between  $V_T$  and  $T_E$  seems to be affected by the available substrate supply. Exercise in glycogen depleted subjects compared with exercise in a control state shifted these plots. Thus, both inspiratory and expiratory time were reduced for an equivalent tidal volume in the glycogen depleted subject. As a result, any given mean inspiratory or expiratory flow rate ( $V_T/T_I$ , or  $V_T/T_E$ ) was achieved by a smaller  $V_T$  and a shorter  $T_I$  or  $T_E$  during ramp exercise in a glycogen depleted state compared with control.



**Fig. 5** The relationship between tidal volume expressed as a percentage of vital capacity ( $V_T$ , %VC) and inspiratory or expiratory duration ( $T_I$  or  $T_E$ , sec) from the onset of incremental cycle ergometer exercise to exhaustion. All data points represent the mean  $\pm$  SEM of both variables.

**VENTILATORY EQUIVALENTS OF OXYGEN CONSUMPTION AND CARBON DIOXIDE PRODUCTION ( $\dot{V}_E/\dot{V}O_2$ ,  $\dot{V}_E/\dot{V}CO_2$ )**

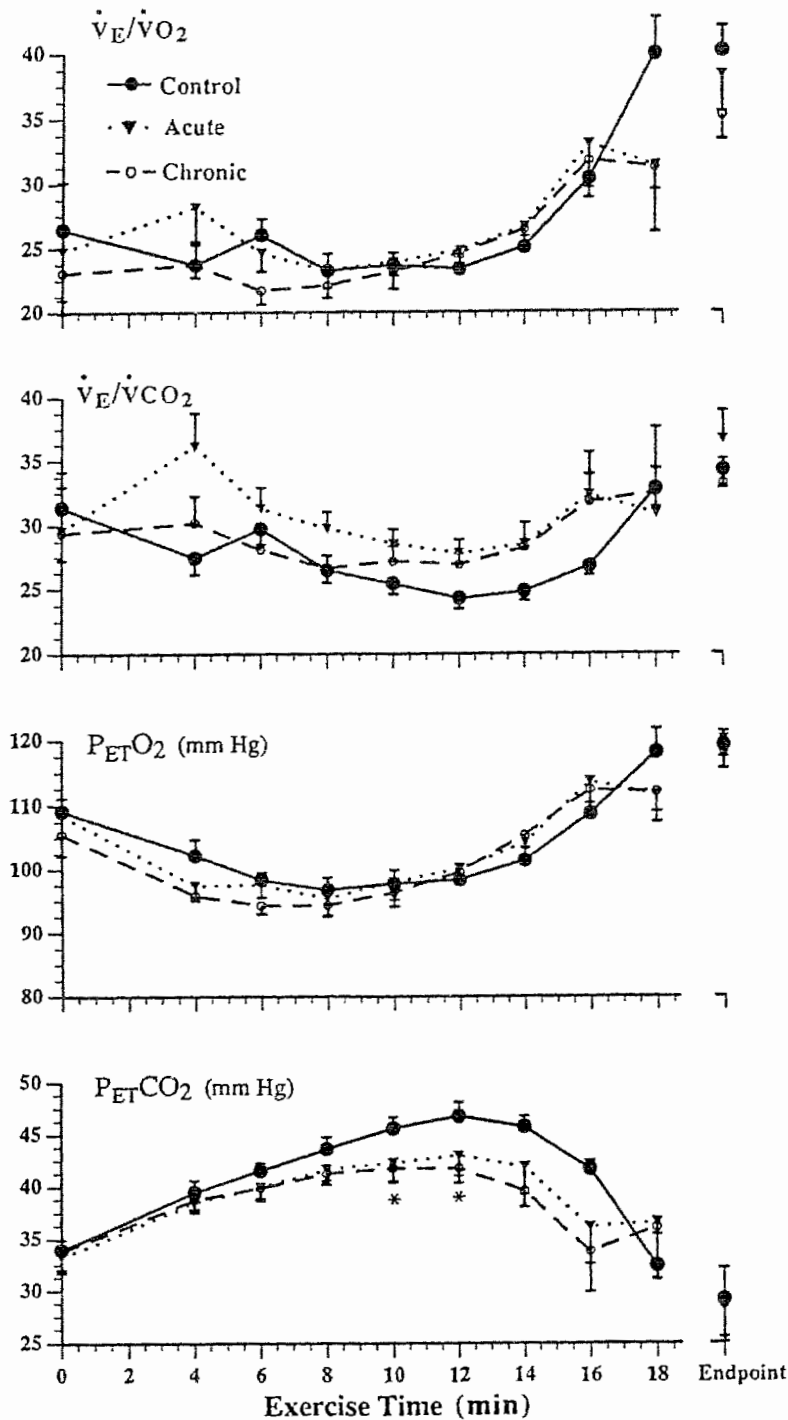
Figure 6 shows the response of  $\dot{V}_E/\dot{V}O_2$ ,  $\dot{V}_E/\dot{V}CO_2$  and end tidal  $PO_2$  and  $PCO_2$  to prolonged exercise. As mentioned previously, at  $t=18$  min in each graph, the data represent an  $n=2$ . The apparent discontinuity in the graphs, therefore, is not representative of the whole group.

The mean ventilatory equivalent for oxygen consumption ( $\dot{V}_E/\dot{V}O_2$ ) was constant in the early phase of incremental exercise in all three exercise trials (Fig. 6). In each condition the group mean  $\dot{V}_E/\dot{V}O_2$  began to increase steeply at the 14th minute of exercise. The ventilatory equivalent for carbon dioxide production ( $\dot{V}_E/\dot{V}CO_2$ ) decreased moderately in the early stages of incremental exercise, then increased as work rate increased (Fig. 6). Visual comparison of the data from each metabolic condition suggested a threshold rise in  $\dot{V}_E/\dot{V}CO_2$ , at the 16th minute in the GN conditions, and at the 14th minute of ramp exercise in the **GD<sub>ACUTE</sub>** and **GD<sub>CHRONIC</sub>** state. However, the difference was not statistically significant between the three conditions.

#### END TIDAL $PO_2$ AND $PCO_2$

End tidal  $PO_2$  decreased at the onset of exercise in both the control and glycogen depleted exercise conditions, then remained constant for several minutes despite an increasing work rate before it finally increased rapidly (Fig. 6). Examination of the mean  $P_{ET}O_2$  suggested that the point of rapid increase in  $P_{ET}O_2$  occurred approximately two minutes earlier (40 to 60 watts earlier) when subjects were in a glycogen depleted state. There was no significant difference in the  $P_{ET}O_2$  observed, however, as a result of glycogen depletion.

The change in  $P_{ET}CO_2$  observed in response to incremental exercise was similar in all glycogen states (Fig. 6). The mean value of  $P_{ET}CO_2$  increased gradually in the early part of exercise, then reached a plateau for a brief period as the work rate continued to increase. After 12 to 14 minutes,  $P_{ET}CO_2$  decreased rapidly as the work rate continued to increase. The mean duration of the plateau phase of  $P_{ET}CO_2$  was not affected by the glycogen depleted state of a subject, but the rapid decline in  $P_{ET}CO_2$  occurred earlier in exercise in both in the **GD<sub>ACUTE</sub>** and **GD<sub>CHRONIC</sub>** exercise. At the 10th and 12th minute of exercise, part of the plateau phase,  $P_{ET}CO_2$  was significantly higher in the GN, compared with either the **GD<sub>ACUTE</sub>** and **GD<sub>CHRONIC</sub>** state.



**Figure 6** Ventilatory equivalent for oxygen uptake and carbon dioxide production ( $\dot{V}_E/\dot{V}O_2$ ,  $\dot{V}_E/\dot{V}CO_2$ ), and the end-tidal  $PO_2$  and  $PCO_2$  ( $P_{ET}O_2$ ,  $P_{ET}CO_2$ ) in response to ramp incremental cycle ergometer exercise to exhaustion. All values are mean  $\pm$  SEM. The group mean data points are expressed as in Fig. 1. \* Significantly different from GN (control) condition at that corresponding time ( $p \leq 0.05$ ).

## HEART RATE

The heart rate response to incremental exercise is shown in Fig. 7. At rest, HR in the **GD<sub>ACUTE</sub>** condition was significantly higher than in either the **GN** or the **GD<sub>CHRONIC</sub>** condition. As expected, HR increased with increasing work intensity; however HR was significantly higher in **GD<sub>ACUTE</sub>** condition compared with both the **GN** or the **GD<sub>CHRONIC</sub>** condition up to and including the 14th minute of exercise. The maximum HR, however, was not significantly different when the three groups were compared at the endpoint, even though the work rate achieved was significantly less as a result of their glycogen depleted condition.

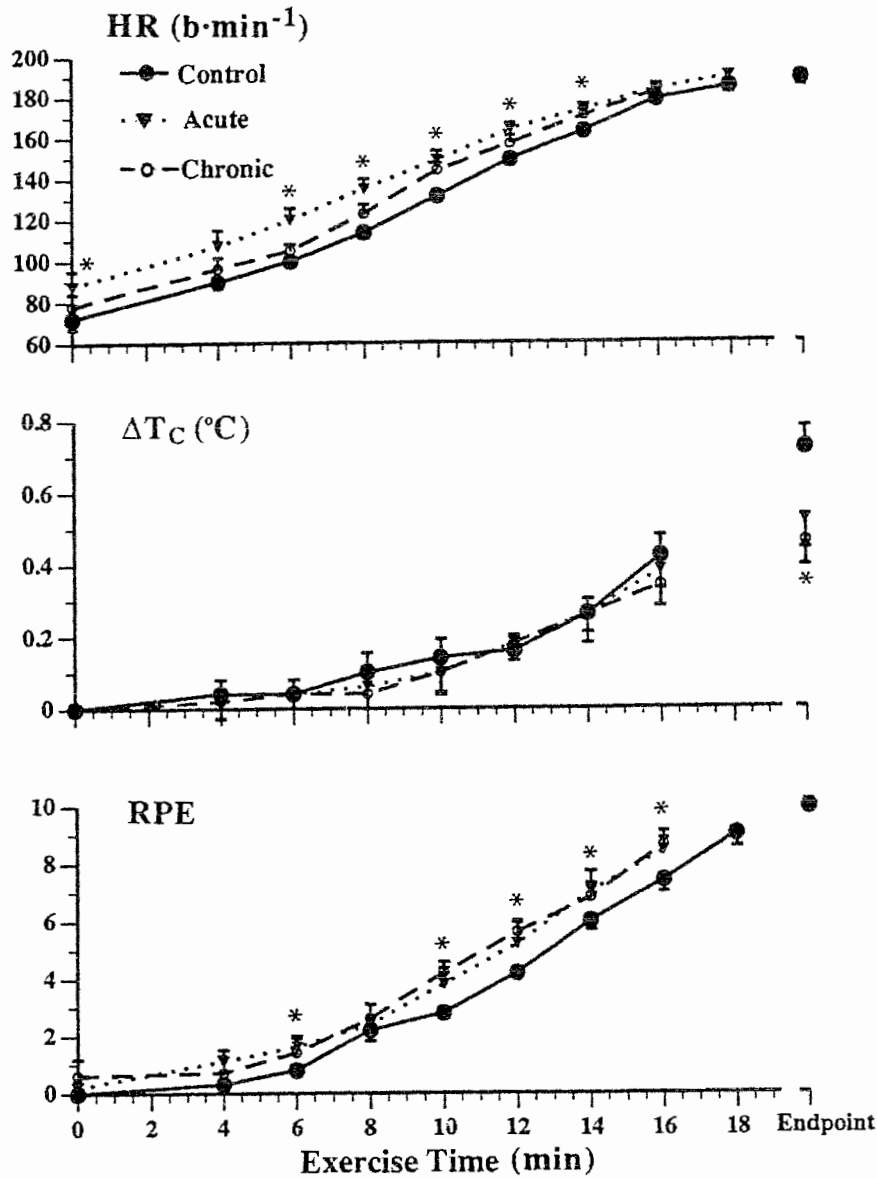
## CORE TEMPERATURE

Ramp incremental exercise to exhaustion produced a similar elevation in a subject's core temperature in all three metabolic conditions (Fig. 7). Expressed as the  $\Delta T_c$  ( $\Delta T_c$ : the difference between core temperature during exercise and core temperature at rest), exhaustive ramp exercise induced a  $0.7 \pm 0.1$  °C elevation in body temperature in the **GN** condition, which was significantly greater than a  $0.5 \pm 0.1$  °C and a  $0.5 \pm 0.1$  °C rise in the **GD<sub>ACUTE</sub>** and **GD<sub>CHRONIC</sub>** states, respectively. Although exercise in the control **GN** condition produced a greater maximum  $\Delta T_c$  between rest and exhaustion, each subject also completed an average of one minute more intense work in the **GN** compared with both glycogen depleted conditions.

## RATING OF PERCEIVED EXERTION

The rating of perceived exertion (RPE) increased in a linear manner in response to ramp incremental exercise to exhaustion (Fig. 7). Based on the RPE, exercise was perceived to be significantly more difficult when a subject was glycogen depleted, at every submaximal work rate except  $t=8$  min, although no significant difference in perceived exertion was observed between the two glycogen depleted states. At exhaustion, RPE was  $9.9 \pm 0.1$  in the **GN** condition, and  $10.0 \pm 0$  in the **GD<sub>ACUTE</sub>** and **GD<sub>CHRONIC</sub>** conditions, respectively.





**Figure 7** The heart rate (HR), rectal temperature ( $\Delta T_C$ ), and the rating of perceived exertion (RPE) in response to ramp incremental cycle ergometer exercise to exhaustion. All values are mean  $\pm$  SEM. The group mean data points are expressed as in Fig. 1. \* Significantly different from GN (control) condition at that corresponding time ( $p \leq 0.05$ ).

## HEMATOCRIT

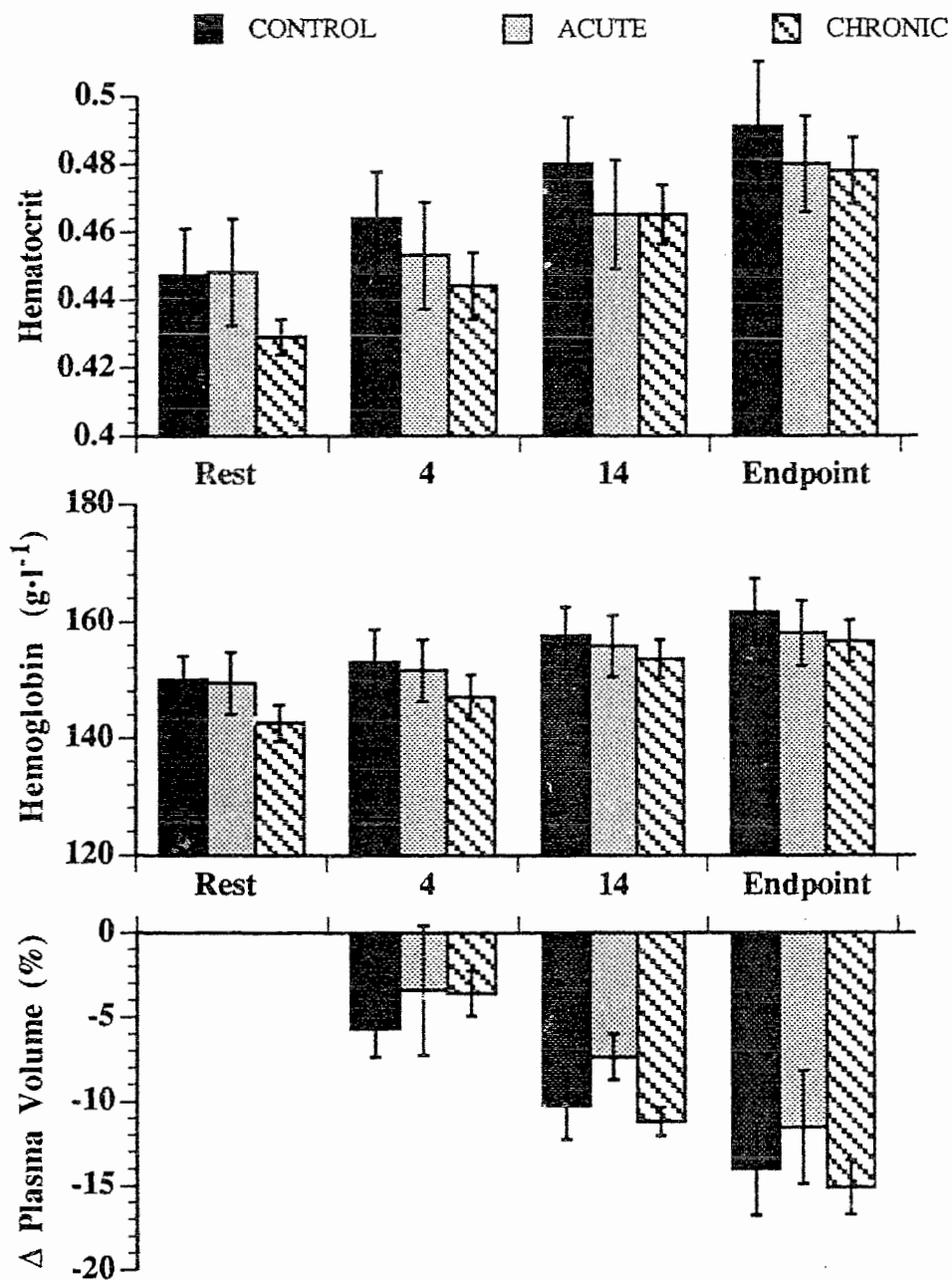
Hemoconcentration during all exercise conditions was reflected by an increased Hct (Fig. 8). The group mean Hct was increased significantly throughout the exhaustive exercise test compared with the resting state, but there was no significant difference between the group means of the **GN**, **GD<sub>ACUTE</sub>**, and **GD<sub>CHRONIC</sub>** conditions. From rest to the exercise endpoint, hematocrit increased from  $0.45 \pm 0.01$  to  $0.49 \pm 0.02$  in the control **GN** condition; from  $0.45 \pm 0.02$  to  $0.48 \pm 0.01$  in the **GD<sub>ACUTE</sub>** exercise; and from  $0.43 \pm 0.01$  to  $0.48 \pm 0.01$  in the **GD<sub>CHRONIC</sub>** state.

## HEMOGLOBIN

The initial resting Hgb concentration was  $150.0 \pm 4.1$ ,  $149 \pm 5.4$ , and  $142.6 \pm 3.1$   $\text{g}\cdot\text{l}^{-1}$  respectively in the **GN**, **GD<sub>ACUTE</sub>**, and **GD<sub>CHRONIC</sub>** conditions, and increased during ramp incremental exercise (Fig. 8). At exercise endpoint, Hgb concentration was  $161.6 \pm 5.7$ ,  $158.0 \pm 5.6$ , and  $156.6 \pm 3.6$   $\text{g}\cdot\text{l}^{-1}$  respectively for the **GN**, **GD<sub>ACUTE</sub>**, and **GD<sub>CHRONIC</sub>** states. The group mean hemoglobin was significantly increased throughout each exhaustive test compared with the resting value within each group, but glycogen depletion did not significantly alter the Hgb relative to the **GN** Hgb value.

## PLASMA VOLUME

Ramp incremental exercise resulted in a decrease in group mean plasma volume from  $56.7 \pm 1.6\%$ ,  $56.7 \pm 1.5\%$ , and  $57.0 \pm 0.5\%$  at rest in the **GN**, **GD<sub>ACUTE</sub>**, and **GD<sub>CHRONIC</sub>** groups respectively, to  $48.9 \pm 2.3\%$ ,  $50.2 \pm 1.2\%$ , and  $48.4 \pm 1.1\%$ , respectively in the same three groups at the highest exercise intensity (Fig. 8). Expressed as a percentage change from rest, plasma volume decreased by  $14.0 \pm 2.7\%$ ,  $11.5 \pm 1.5\%$ , and  $15.1 \pm 1.6\%$  in the **GN**, **GD<sub>ACUTE</sub>**, and **GD<sub>CHRONIC</sub>** states. The decrease was significantly different from the resting control value within each group, but not significantly different between exercise conditions.



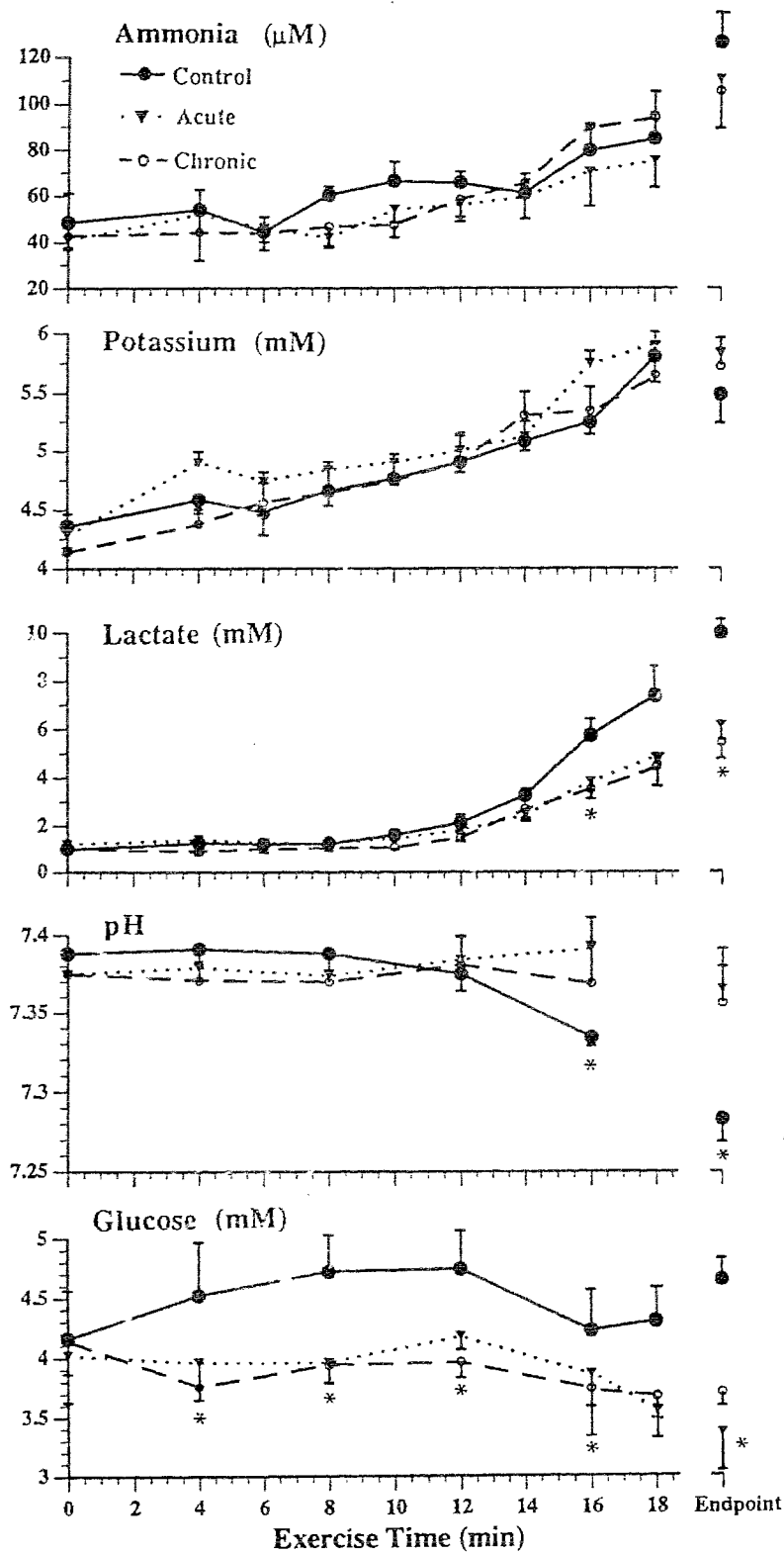
**Figure 8** The group mean change in hematocrit (Hct), hemoglobin (Hgb), and plasma volume ( $\Delta$ PV) in response to ramp incremental cycle ergometer exercise to exhaustion. Values are means  $\pm$  SEM for 5 subjects.

## BLOOD METABOLITES

Fig. 9 shows the group mean change in the concentration of blood ammonia ( $[\text{NH}_3]$ ), potassium ( $[\text{K}^+]$ ), lactate ( $[\text{La}]$ ), pH, and glucose during ramp incremental exercise to exhaustion. Where appropriate in the text, the absolute value of the metabolites is compared to the concentration corrected for any plasma volume shift during exercise. As a result of the marked hemoconcentration and decrease in plasma volume observed with increased exercise intensity, the corrected metabolite concentration represents an effective dilution of the metabolite.

### AMMONIA

At rest, the group mean pre-exercise venous ammonia concentration ( $\text{NH}_3$ ) was  $48.5 \pm 12.6 \mu\text{M}$ ,  $40.8 \pm 3.9 \mu\text{M}$ , and  $42.5 \pm 4.9 \mu\text{M}$  at rest in the **GN**, **GD<sub>ACUTE</sub>**, and **GD<sub>CHRONIC</sub>** metabolic states respectively (Fig. 9).  $\text{NH}_3$  concentration increased gradually during incremental exercise to  $125.7 \pm 14.5 \mu\text{M}$ ,  $109.9 \pm 20.6 \mu\text{M}$ , and  $105.1 \pm 16 \mu\text{M}$  in the **GN**, **GD<sub>ACUTE</sub>**, and **GD<sub>CHRONIC</sub>** conditions at the endpoint of exercise. A further increase in venous  $\text{NH}_3$  concentration was observed during the early stage of recovery in both the **GN** and **GD<sub>CHRONIC</sub>** conditions, when a peak  $\text{NH}_3$  concentration of  $165.6 \pm 27.7$  and  $119.4 \pm 19.7 \mu\text{M}$  was reached in the respective **GN** and **GD<sub>CHRONIC</sub>** state. In the **GD<sub>ACUTE</sub>** condition, the peak ammonia concentration was observed at the endpoint of exercise. During exhaustive exercise in all three metabolic conditions, the group mean blood ammonia concentration was consistently and significantly elevated from rest after the 12th minute of incremental exercise. However, no significant difference was determined in blood ammonia concentration between the control and glycogen depleted groups at any time point during exhaustive exercise. Correction of the blood ammonia concentration for a plasma volume shift during incremental exercise did not affect the statistical significance in the group mean ammonia concentration between or within groups reported above.



**Figure 9** Blood metabolite changes (ammonia,  $[\text{NH}_3]$ ; potassium,  $[\text{K}^+]$ ; lactate,  $[\text{La}]$ ; pH; Glucose) in response to ramp incremental cycle ergometer exercise to exhaustion. All values are mean  $\pm$  SEM. The group mean data points are expressed as in Fig. 1.  
 \* Significantly different from GN (control) condition at that corresponding time ( $p \leq 0.05$ ).

## POTASSIUM

Resting group mean venous potassium concentration ( $[K^+]$ ) was  $4.4 \pm 0.2$ ,  $4.3 \pm 0.2$ , and  $4.1 \pm 0.1$  in the **GN**, **GD<sub>ACUTE</sub>**, and **GD<sub>CHRONIC</sub>** conditions respectively (Fig. 9). Within each group,  $[K^+]$  increased significantly from rest in response to unloaded pedalling, and continued to increase steadily and significantly compared with the resting concentration throughout exercise. No significant difference in the response of  $[K^+]$  to exercise was observed between the three groups. The peak concentration of potassium, observed at the endpoint of exercise, was  $5.5 \pm 0.2$  mM,  $5.8 \pm 0.1$  mM, and  $5.7 \pm 0.1$  mM for **GN**, **GD<sub>ACUTE</sub>**, and **GD<sub>CHRONIC</sub>** groups, respectively. When  $[K^+]$  was corrected ( $[K^+]_{COR}$ ) for the plasma volume shift,  $[K^+]_{COR}$  was consistently and significantly greater in the **GD<sub>ACUTE</sub>** state than in either the **GN** or the **GD<sub>CHRONIC</sub>** groups.

## LACTATE

Altering the glycogen state of a subject resulted in a significant reduction in the venous lactate response to ramp exercise (Fig. 9). Resting venous lactate concentration ( $[La]$ ) was not different in glycogen depleted subjects. In all three metabolic conditions, a curvilinear increase in  $[La]$  was observed as exercise intensity increased. The exercise-induced increase in the group mean  $[La]$  was significantly greater in the **GN** state compared with both glycogen depleted conditions from the 16th minute of exercise onwards. At the endpoint of exercise, the group mean  $[La]$  was  $10.0 \pm 0.6$  mM,  $6.1 \pm 0.6$  mM, and  $5.4 \pm 0.6$  mM in the **GN**, **GD<sub>ACUTE</sub>** and **GD<sub>CHRONIC</sub>** conditions, respectively. The peak venous  $[La]$  of  $12.1 \pm 0.7$  mM was observed at the 5th recovery minute in the **GN** group. In the **GD<sub>ACUTE</sub>** and the **GD<sub>CHRONIC</sub>** condition, the peak lactate concentration was observed 2.5 minutes into recovery, slightly earlier than the time point of peak blood lactate concentration for the **GN** group. Peak lactate concentration was  $7.1 \pm 0.6$  mM in **GD<sub>ACUTE</sub>** and  $7.6 \pm 0.7$  mM the **GD<sub>CHRONIC</sub>** conditions, respectively. Correction of venous lactate concentration for plasma

shifts during exercise did not change the statistical relationship of the metabolite between groups.

## pH

pH, which was measured every 4th minute during ramp incremental exercise, showed a marked effect of glycogen depletion. A greater acidosis was observed during exercise in the GN state compared with either glycogen depleted conditions (Fig. 9). The group mean pH was the same in the three exercise groups at rest ( $7.39 \pm 0.01$  for GN,  $7.38 \pm 0.02$  for GD<sub>ACUTE</sub>, and  $7.38 \pm 0.01$  for GD<sub>CHRONIC</sub>), and in the early stages of exercise. In the GN condition, pH decreased dramatically after the 12th minute of exercise. In contrast, the blood pH remained essentially constant throughout the exhaustive exercise protocol in the two glycogen depleted conditions. Only a small decrease in pH at the point of exhaustion was observed in each subject. At the endpoint of GN exercise, the group mean pH was  $7.28 \pm 0.01$  and continued to decrease to a minimum value of  $7.21 \pm 0.01$  by the 5th minute of recovery, compared with a pH minimum of  $7.34 \pm 0.02$  and  $7.31 \pm 0.02$  observed in GD<sub>ACUTE</sub> and GD<sub>CHRONIC</sub> conditions, respectively. From the 16th minute of exercise, the group mean pH was significantly lower in the GN condition compared with the two glycogen depleted states.

## GLUCOSE

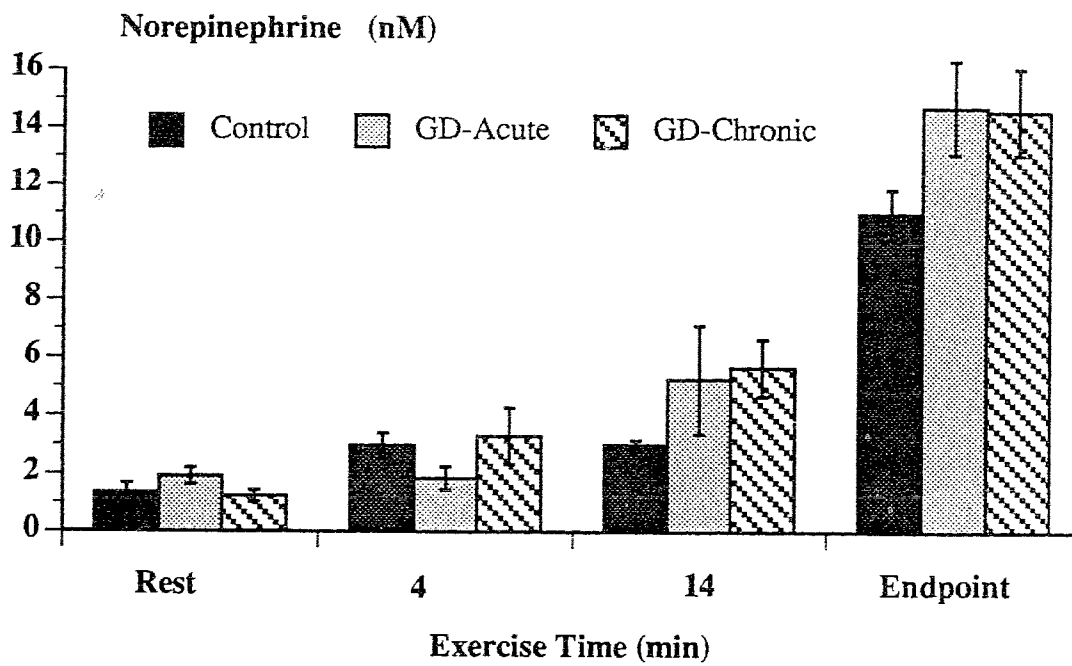
At rest, blood glucose concentration was not affected significantly by prior glycogen depletion. Throughout ramp incremental exercise, however, depletion of muscle glycogen of an individual prior to exercise resulted in a significant reduction in blood glucose concentration (Fig. 9). Blood glucose tended to rise in the GN condition in the early stage of the exercise test, whereas in the glycogen depleted state, blood glucose tended to decrease from the onset of exercise. At the endpoint of exercise, the blood glucose concentration was  $4.6 \pm 0.2$  mM in

the GN condition, compared with  $3.4 \pm 0.4$  and  $3.7 \pm 0.1$  in the GD<sub>ACUTE</sub> and GD<sub>CHRONIC</sub> conditions, respectively. When blood glucose concentration was corrected for the shift in plasma volume observed with exercise, there was no longer a significant difference between the GN and GD<sub>ACUTE</sub> conditions; however the difference between the GN and the GD<sub>CHRONIC</sub> state remained significant.

#### NOREPINEPHRINE

Plasma norepinephrine concentration was determined at rest, during unloaded pedalling exercise, at the 14th minute, and at the endpoint of each exercise session. During exercise in the GD<sub>ACUTE</sub> condition, it was not possible to obtain a blood sample for catecholamine analysis in one subject due to problems with the catheter, and data at the 4th minute, 14th minute, and endpoint represent the mean of 4 subjects only. Plasma norepinephrine increased significantly from its resting concentration in response to ramp incremental exercise in all three metabolic conditions (Fig. 10). At rest, the group mean plasma norepinephrine concentration was  $1.3 \pm 0.3$  nM in the GN,  $1.9 \pm 0.3$  nM in the GD<sub>ACUTE</sub>, and  $1.2 \pm 0.2$  nM in the GD<sub>CHRONIC</sub> state. During exercise in the GD<sub>ACUTE</sub> and GD<sub>CHRONIC</sub> state, the group mean plasma norepinephrine concentration tended to be greater than in the GN condition, but the large inter-subject variation prevented detection of any significant difference. At the endpoint of exhaustive exercise, the group mean norepinephrine concentration was  $11.0 \pm 0.8$  nM,  $14.7 \pm 1.6$  nM, and  $14.6 \pm 1.5$  nM in the GN, GD<sub>ACUTE</sub> and GD<sub>CHRONIC</sub> state, respectively.





**Figure 10** Plasma norepinephrine in response to ramp incremental cycle ergometer exercise to exhaustion. Values are means  $\pm$  SEM for n=5 subjects, except in the  $GD_{ACUTE}$  condition, where n=4 at 4 min, 14 min, and Endpoint.

## RELATIONSHIP BETWEEN VENTILATION AND MEDIATORS OF VENTILATION DURING RAMP EXERCISE

Fig. 11 shows the inter-relationship between the response of ventilation ( $V_e$ ) and breathing frequency to ramp incremental exercise, compared with several potential mediators of the ventilatory response. Venous ammonia, potassium, pH, norepinephrine, and core temperature ( $\Delta T_C$ ), which have been individually related to the stimulation of ventilation, are compared. Individual scattergrams showing the relationship between the change in ventilation and each potential mediator of ventilation in response to ramp incremental exercises are shown in Appendix IV, Fig. 24-38.

Ventilation, which increased in response to the incremental ramp protocol, was significantly greater at 14 and 16 minutes of exercise when subjects were glycogen depleted, but was not significantly greater at exhaustion compared with the control condition. In the control condition, however, the group mean exercise time to exhaustion was  $18.5 \pm 0.4$  min, compared with  $17.5 \pm 0.4$  and  $17.5 \pm 0.5$  min in the  $GD_{ACUTE}$  and  $GD_{CHRONIC}$  conditions, respectively. At the time when ventilation was significantly greater in the glycogen depleted condition, breathing frequency ( $f$ ) tended also to be greater. The tachypneic response observed in the glycogen depleted subject, although not significantly greater than in the control condition, was still sufficient to increase ventilation significantly, since tidal volume showed no upward trend at this time (Fig. 7).

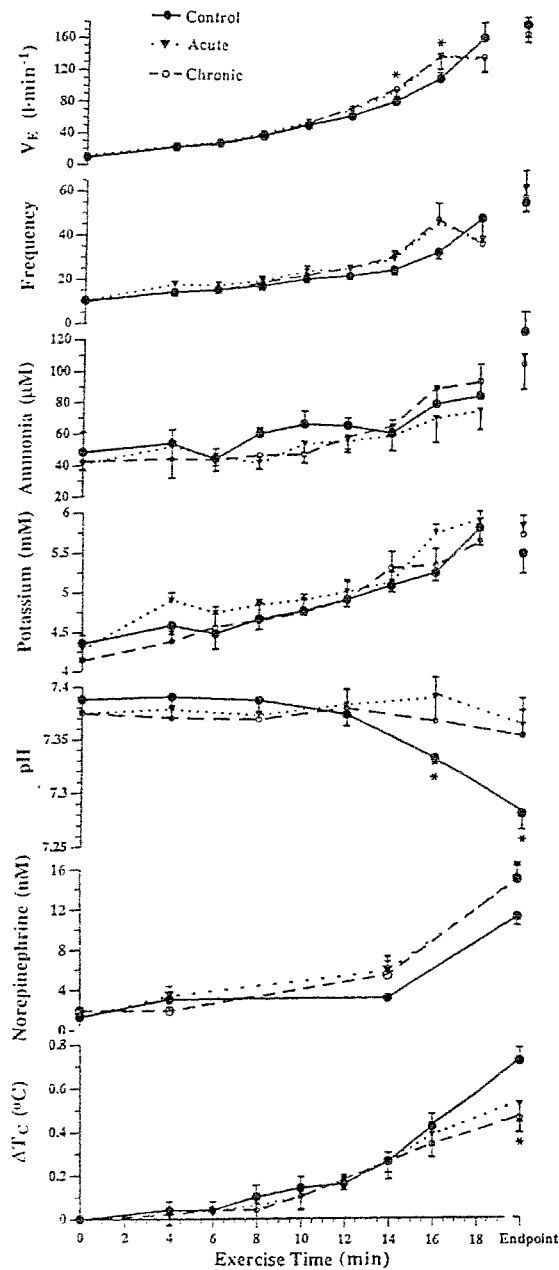
Venous ammonia concentration [ $NH_3$ ], which increased in response to ramp exercise, was not significantly different in the glycogen depleted, compared with the control condition. The significant increase in ventilation during exercise in the glycogen depleted condition did not correspond with a consistent increase in [ $NH_3$ ], although the rate of increase in hyperammonemia seemed higher as exercise progressed. Venous potassium [ $K^+$ ] concentration, which increased steadily throughout exercise, also showed no significant difference in the control and glycogen depleted conditions. The tendency of [ $K^+$ ] to increase rapidly at 18 min, then decrease at Endpoint, particularly in the control condition, reflected the progressively smaller number of subjects ( $GN$ ,  $n=4$ ;  $GD_{ACUTE}$  and

GD<sub>CHRONIC</sub>, n=2) who were able to continue to this point. This discontinuity in the group mean  $[K^+]$  response is not matched by an equivalent deviation in  $\dot{V}_E$ , compared with the Endpoint value.

The response of venous pH (measured every 4 minutes) to ramp incremental exercise in the GN condition remained unchanged initially, then decreased progressively after 12 minutes of exercise to the Endpoint. However, no equivalent decrease in pH was observed in either of the glycogen depleted states. The timing of the decrease in pH corresponded with a non-linear increase in ventilation in the control, GN condition. Thus, at equivalent time points in the glycogen depleted states, a relative alkalosis was present. The absence of a fall in pH in the glycogen depleted states, suggests that pH did not contribute to the significant increase in ventilation observed in this condition.

The profile of the change in norepinephrine concentration, measured at 4 min, 14 min, and at the endpoint of ramp incremental exercise, shows a similar pattern of increase to ventilation. The small number of norepinephrine samples, however, precludes a stringent comparison of the change in this potential mediator with the ventilatory data.

Fig. 11 shows the progressive increase in core temperature as  $\Delta T_C$ , which increased progressively in response to ramp exercise. The absolute increase in core temperature was significantly smaller, however, at the endpoint of exercise in the glycogen depleted subjects (  $0.5 \pm 0.1$  °C in both the GD<sub>ACUTE</sub> and GD<sub>CHRONIC</sub> conditions) compared with an increase of  $0.7 \pm 0.1$  °C in the GN condition. At the equivalent time when a significantly higher ventilation was observed in the glycogen depleted state, core temperature was similar to the control condition. However, it was significantly lower at the exhaustive endpoint of exercise. As previously noted, this may reflect the duration of exercise, which was significantly longer in the GN condition.



**Fig. 11** Effects of ramp incremental exercise on minute ventilation ( $V_E$ ), breathing frequency, venous ammonia, potassium, pH, norepinephrine, and change in core temperature ( $\Delta T_c$ ). All values are means  $\pm$  SEM. \* indicates significantly different from the GN (control) condition.

**CORRELATION OF RATING OF PERCEIVED EXERTION WITH PHYSIOLOGICAL AND BIOCHEMICAL MEASURES DURING RAMP INCREMENTAL EXERCISE**

During ramp incremental exercise, the group mean RPE increased in parallel with the ramp work rate (Fig. 7), although RPE was significantly greater during exercise in both the  $GD_{ACUTE}$  and  $GD_{CHRONIC}$  conditions. Table 5 shows that RPE measured during exercise in the GN condition correlates strongly with several physiological and biochemical measurements. In all 3 exercise conditions, the strong correlation of RPE with  $\Delta\dot{V}_E$  and  $\Delta HR$  is most consistent, although the relationship with  $\Delta[NH_3]$ ,  $\Delta[K^+]$ ,  $\Delta[NE]$ ,  $\Delta[La]$  and  $\Delta T_c$  is relatively consistent between each condition. RPE show a strong negative correlation with  $\Delta pH$  only in the GN condition.

**Table 5** The Pearson correlation coefficient of the change in RPE ( $\Delta RPE$ ) versus  $\Delta\dot{V}_E$ ,  $\Delta[NH_3]$ ,  $\Delta[K^+]$ ,  $\Delta pH$ ,  $\Delta[La]$ ,  $\Delta HR$ ,  $\Delta[NE]$ ,  $\Delta T_c$ , and  $\Delta$  Glucose in response to ramp incremental cycle ergometer exercise. The delta ( $\Delta$ ) values were calculated as the difference from rest in each variable at a specific time point. The correlation coefficients were calculated from data collected during the time period indicated. In each condition, the correlation coefficients were determined for exercise from 4 minutes to Endpoint. The number in parentheses indicates the (n) for each correlation coefficient.

Condition	$\Delta\dot{V}_E$	$\Delta[NH_3]$	$\Delta[K^+]$	$\Delta pH$	$\Delta[La]$	$\Delta HR$	$\Delta[NE]$	$\Delta T_c$	$\Delta$ Glucose
GN	0.93 (45)	0.62 (45)	0.67 (45)	-0.77 (25)	0.89 (45)	0.92 (45)	0.74 (15)	0.86 (45)	-0.14 (25)
$GD_{ACUTE}$	0.84 (40)	0.57 (40)	0.77 (40)	-0.12 (25)	0.74 (40)	0.82 (40)	0.80 (12)	0.76 (40)	-0.32 (25)
$GD_{CHRONIC}$	0.84 (40)	0.72 (40)	0.70 (40)	-0.26 (25)	0.76 (40)	0.82 (40)	0.72 (15)	0.61 (40)	-0.29 (25)

## SUMMARY: RESULTS OF RAMP EXERCISE

Ramp incremental exercise to exhaustion resulted in a characteristic increase in ventilation and gas exchange. In the early stage of the ramp,  $\dot{V}_E$ ,  $\dot{V}O_2$ , and  $\dot{V}CO_2$  increased in proportion to the metabolic demand of exercise. In the later, more intense phase of the ramp, the time course of  $\dot{V}_E$  and  $\dot{V}CO_2$  accelerated and deviated from the linear metabolic response. Time to exhaustion (and therefore maximum work rate) was also significantly shortened during exercise in both the **GD<sub>ACUTE</sub>** and **GD<sub>CHRONIC</sub>** condition compared with the control, **GN** state.  $\dot{V}_E$  was significantly greater during the intense, but less than maximal phase of exercise in the glycogen depleted states compared with the control condition, although the maximum  $\dot{V}_E$  was not significantly different.

The relative hyperventilation observed in the glycogen depleted subjects resulted primarily from an increase in breathing frequency.  $V_T/T_I$ , assumed to reflect ventilatory drive, was significantly greater at high work rates during exercise in the glycogen depleted state compared with the control condition. Analysis of the  $V_T$ - $T_I$ - $T_E$  diagram showed that for any given tidal volume, both inspiratory and expiratory time were shorter in glycogen depleted, compared with control subjects. As a result, any given mean inspiratory or expiratory flow rate ( $V_T/T_I$ , or  $V_T/T_E$ ) was achieved by a smaller  $V_T$  and a shorter  $T_I$  or  $T_E$  during exercise in glycogen depleted, compared with control conditions.

Ramp incremental exercise produced an elevation in venous ammonia, potassium, norepinephrine and lactate in both the control and the glycogen depleted conditions. Blood lactate concentration was significantly reduced during exercise in the glycogen depleted state relative to the control condition, but the respective concentration of venous ammonia, potassium, and norepinephrine were not significantly altered by prior glycogen depletion of the subjects. The progressive metabolic acidosis observed in the control exercise condition, was completely attenuated in glycogen depleted subjects. Core temperature, which rose in response to exercise, was not significantly different when a subject was glycogen depleted.

Glycogen depletion significantly increased a subject's perception of effort during ramp incremental exercise. Several of the potential mediators of ventilation, as well as  $\Delta\dot{V}_E$ , correlated strongly with perception of effort (RPE) during ramp exercise. While the time dependent nature of these variables is recognized, and no cause-and-effect relationship is implied by the analysis, these observations provide interesting insight into the physiological and biochemical factors which may contribute to perceived exertion during exercise.

## THE PHYSIOLOGICAL RESPONSE TO PROLONGED EXERCISE

The ventilatory response to prolonged steady state exercise was examined during exercise in a **GN** and a **GD<sub>ACUTE</sub>** condition, and compared with several potential mediators of ventilation. 5 male subjects performed submaximal exercise at 50% of the  $WR_{max}$  determined in the preliminary ramp incremental test (Table 3). During prolonged exercise, endpoint of exercise was defined as the point at which each subject could no longer maintain the pedalling frequency at an assigned work rate, or 90 minutes after the step increase in work rate.

The graphic representation of group mean data in the **GN** condition represent data for n=5 subjects at all time points up to 50 minutes of exercise, and n=4 for the time points from 55 to 90 minutes. In the **GD<sub>ACUTE</sub>** condition, all 5 subjects were able to complete 25 minutes of exercise, and the group mean data represent an n=5 up to that point. The Endpoint also represents n=5 subjects in both treatments. The graphs were plotted up to 25 minutes of exercise and including the endpoint, to avoid the impression of discontinuity because of the difference in the number subjects at each data point. Also, to include all subjects in the analysis, statistical comparison of the **GN** and **GD<sub>ACUTE</sub>** data by ANOVA could only be done up to 25 minutes of exercise, and at the endpoint of exercise.

### WORK DURATION

The mean exercise time for prolonged steady state exercise at 50%  $WR_{MAX}$  was reduced from  $82.0 \pm 8.0$  minutes in the control **GN** condition to  $33.4 \pm 4.5$  minutes in the **GD<sub>ACUTE</sub>** condition, and averaged  $43.3 \pm 8.3\%$  of the control exercise duration. In the control **GN** condition, one subject stopped exercise at 50 minutes, which included 5 minutes of unloaded pedalling and 45 minutes at 50%  $WR_{max}$ . The remaining four subjects completed 95 minutes of exercise, including 5 minutes of unloaded pedalling at the onset of exercise. In the **GD<sub>ACUTE</sub>** condition, two subjects were unable to continue beyond 25 minutes, while one stopped at 30, 35, and 50 minutes.

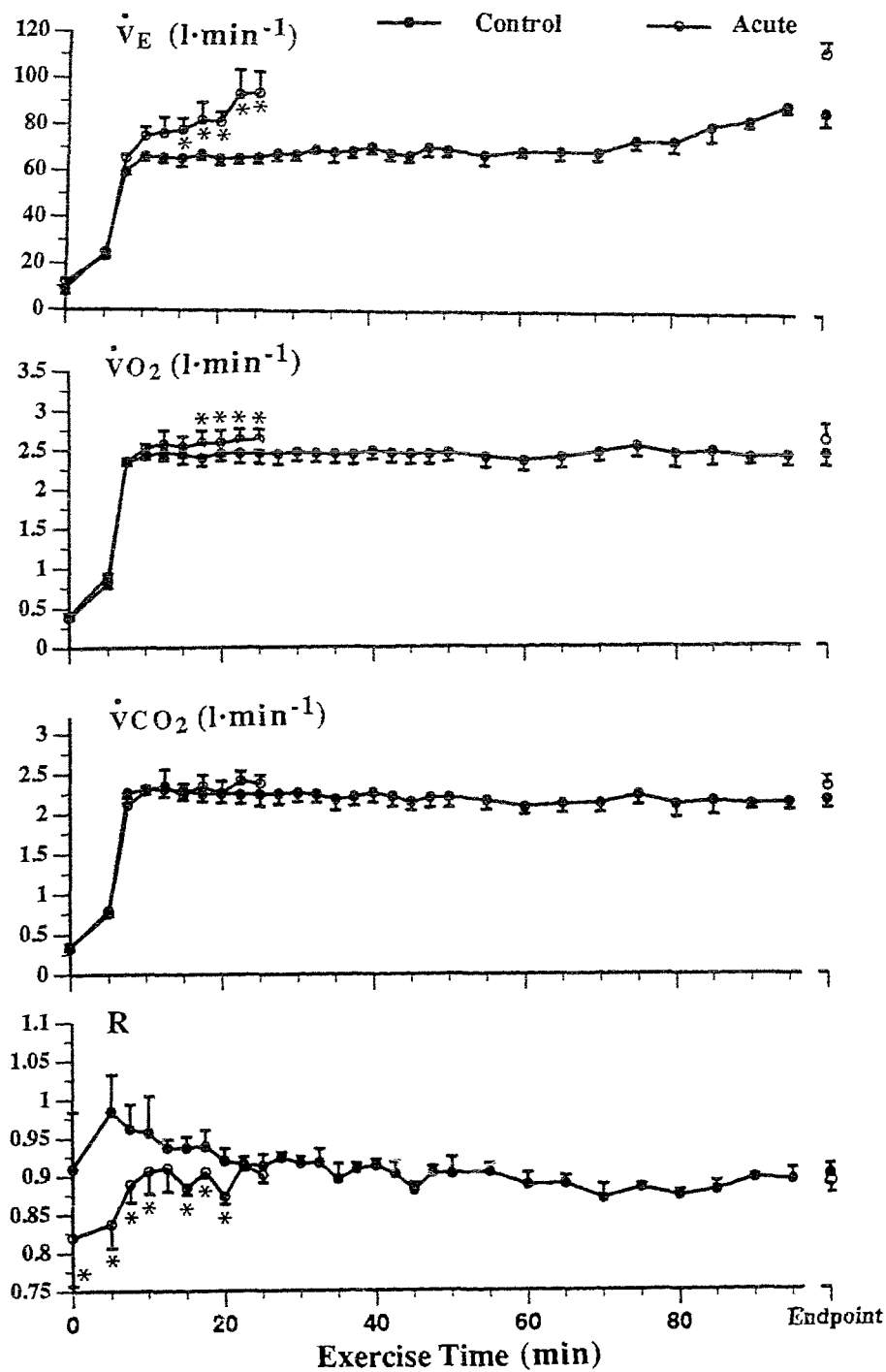


## VENTILATION

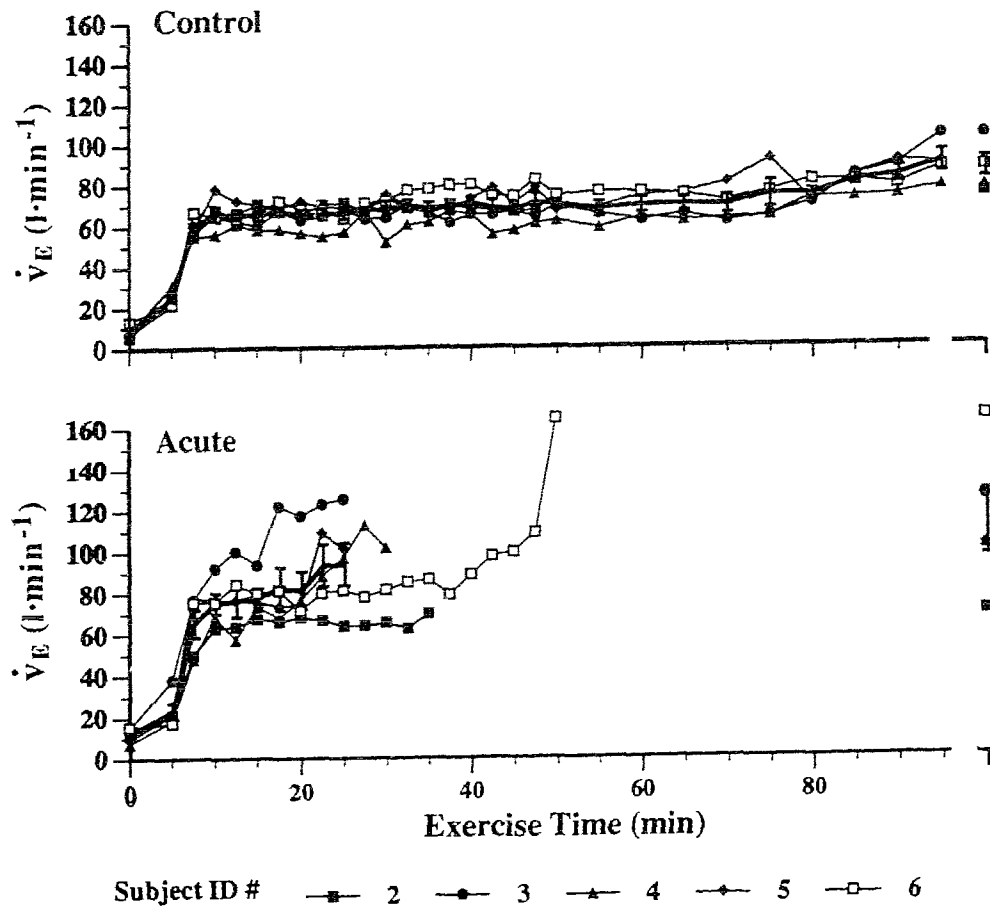
The group mean ventilatory response to prolonged exercise is shown in Fig. 12 compared with other gas exchange variables. The individual ventilatory response to prolonged exercise at 50%  $WR_{max}$  in relation to the group mean  $\dot{V}_E$  is shown in Fig. 13.

A small increase in minute ventilation ( $\dot{V}_E$ ) was observed between rest and unloaded pedalling (5 min), followed by a rapid increase in  $\dot{V}_E$  to a steady state value when 50%  $WR_{max}$  load was applied (Fig. 12). The  $\dot{V}_E$  at rest in the **GN** condition was  $9.0 \pm 1.4$  l., rising to a steady state value of  $66.0 \pm 3.5$  l. by the 10th minute of exercise which was then remarkably constant until approximately 70 minutes of exercise. A gradual upward drift in ventilation from this time resulted in an endpoint  $\dot{V}_E$  of  $86.7 \pm 5.1$  l. in the **GN** condition.

Glycogen depletion of a subject significantly affected the ventilatory response to a step increase in work rate. The group mean  $\dot{V}_E$  was similar at rest (**GN** =  $9.0 \pm 1.4$  l·min<sup>-1</sup> and **GD<sub>ACUTE</sub>** =  $12.0 \pm 1.6$  l·min<sup>-1</sup>) and during unloaded pedalling (**GN** =  $24.7 \pm 1.8$  l·min<sup>-1</sup> and **GD<sub>ACUTE</sub>** =  $23.1 \pm 3.8$  l·min<sup>-1</sup>) in the **GN** and **GD<sub>ACUTE</sub>** conditions, respectively. However, once the work rate increased to 50%  $WR_{max}$ , the group mean  $\dot{V}_E$  was consistently higher in the **GD<sub>ACUTE</sub>** compared to the **GN** condition, and this difference was significant from 15 min of exercise. The mean  $\dot{V}_E$  also drifted upwards from the onset of the step increase in the work rate in the **GD<sub>ACUTE</sub>** state, rather than attaining a prolonged plateau as was observed in the **GN** condition. The maximum difference was observed at the point of exhaustion, when the group mean  $\dot{V}_E$  was significantly higher at  $86.7 \pm 5.1$  l·min<sup>-1</sup> in the **GN** state compared with  $112.4 \pm 15.7$  l·min<sup>-1</sup> in the **GD<sub>ACUTE</sub>** condition.



**Fig. 12** Ventilatory and gas exchange responses ( $\dot{V}_E$ ,  $\dot{V}O_2$ ,  $\dot{V}CO_2$ , and R) to prolonged cycle ergometer exercise at 50%  $WR_{max}$ . All values are mean  $\pm$  SEM. In the GN treatment, n=5 until 50 min. and at the Endpoint, and n=4 between 55 and 95 min. In the  $GD_{ACUTE}$  treatment, n=5. The **Endpoint** value represents the group mean value at the endpoint of exercise, irrespective of the total exercise duration of each subject. \* Significantly different from GN (control) condition at that corresponding time ( $p \leq 0.05$ ).



**Fig. 13** The individual ventilatory response ( $\dot{V}_E$ ) to prolonged cycle ergometer exercise at 50%  $WR_{max}$  in relation to the group mean  $\dot{V}_E$ . Data for each individual subject are indicated by a subject ID number. The group mean data points are expressed as in Fig. 12.

### OXYGEN CONSUMPTION

Glycogen depletion did not significantly affect the  $\dot{V}O_2$  observed at rest or during unloaded pedalling. However, a step increment in work rate from unloaded pedalling to an individual's 50%  $WR_{max}$  produced a rapid rise in  $\dot{V}O_2$ , and the group mean steady state  $\dot{V}O_2$  was significantly from 17.5 minutes of exercise in the  $GD_{ACUTE}$  condition compared with exercise in the GN state (Fig. 12). At the point of exhaustion, the group mean  $\dot{V}O_2$  was  $2.40 \pm 0.12$  l·min<sup>-1</sup> in the GN condition compared with  $2.57 \pm 0.15$  l·min<sup>-1</sup> in the  $GD_{ACUTE}$  condition.

## CARBON DIOXIDE PRODUCTION

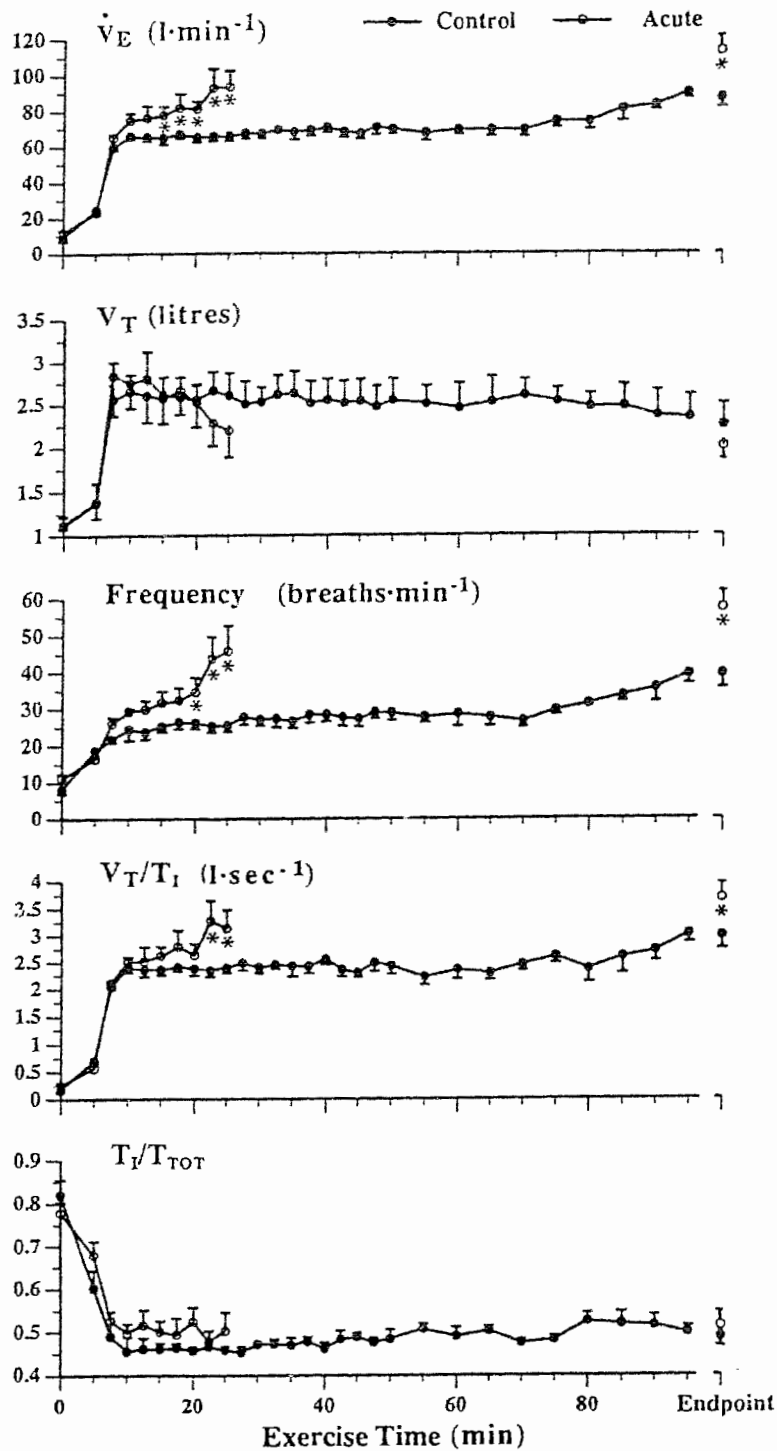
$\dot{V}CO_2$  was not different between control and acute glycogen deleted subjects either at rest or during unloaded pedalling (Fig. 12). At the onset of the step increase in work rate,  $\dot{V}CO_2$  rose with a rapid exponential response in a similar manner to that observed for  $\dot{V}O_2$ . The difference in  $\dot{V}CO_2$  between the **GN** and **GD<sub>ACUTE</sub>** exercise was not significant throughout exercise. At exhaustion, the group mean  $\dot{V}CO_2$  was  $2.15 \pm 0.09$  l·min<sup>-1</sup> and  $2.32 \pm 0.17$  l·min<sup>-1</sup> in **GN** and **GD<sub>ACUTE</sub>** conditions respectively.

## RESPIRATORY EXCHANGE RATIO

The respiratory exchange ratio ( $R = \dot{V}CO_2 / \dot{V}O_2$ ) reflected the effect of glycogen depletion on substrate utilization. At rest the group mean R was  $0.91 \pm 0.03$  in the **GN** condition and  $0.82 \pm 0.02$  in **GD<sub>ACUTE</sub>** condition (Fig. 12). During exercise in both the **GN** and **GD<sub>ACUTE</sub>** condition, R increased steeply at the onset of exercise to a peak value of  $0.98 \pm 0.05$  in the **GN** condition and 0.91 in the **GD<sub>ACUTE</sub>** condition, then gradually declined as the steady state submaximal exercise continued. At the endpoint of exercise, an R value of 0.90 was observed in both groups. The group mean R value was significantly lower up to the 20th minute of exercise in the **GD<sub>ACUTE</sub>** state compared with the **GN** condition.

## COMPONENTS OF VENTILATION

The output of the respiratory system was analyzed in terms of pulmonary ventilation ( $\dot{V}_E$ ) and its two traditional components, tidal volume ( $V_T$ ) and ventilatory frequency ( $f$ ), as well as components of ventilation proposed by Milic-Emili and Grunstein (1976), inspiratory drive ( $V_T/T_I$ ) and inspiratory timing ( $T_I/T_{TOT}$ ). A comparison of  $\dot{V}_E$ ,  $V_T$ ,  $f$ ,  $V_T/T_I$ , and  $T_I/T_{TOT}$  at each work rate during prolonged steady state exercise is shown in Fig. 14.



**Figure 14** Pulmonary ventilation ( $\dot{V}_E$ ) and the components of ventilation ( $V_T$ ,  $f$ ,  $V_T/T_I$ ,  $T_I/T_{TOT}$ ) in response to prolonged cycle ergometer exercise at 50%  $WR_{max}$ . All values are mean  $\pm$  SEM. The group mean data points are expressed as in Fig. 12.  
 \* Significantly different from GN (control) condition at that corresponding time ( $p \leq 0.05$ ).

## TIDAL VOLUME

The response of tidal volume ( $V_T$ , litres) to prolonged exercise is shown in Fig. 14. Glycogen depletion did not significantly affect a subject's tidal volume ( $V_T$ ) at rest, (**GN** =  $1.11 \pm 0.17$  l versus **GD<sub>ACUTE</sub>** =  $1.13 \pm 0.19$  l) or in response to unloaded pedalling (**GN** =  $1.38 \pm 0.10$  l versus **GD<sub>ACUTE</sub>** =  $1.39 \pm 0.20$  l). In both the **GN** and **GD<sub>ACUTE</sub>** condition,  $V_T$  increased rapidly with the onset of a 50 %  $WR_{MAX}$  step increment in work rate. However, as exercise progressed, there was a gradual decrease in the group mean  $V_T$  from the onset of the step increment in work rate to the endpoint of prolonged steady state exercise.

Unlike  $\dot{V}_E$ , which was greater during exercise in the **GD<sub>ACUTE</sub>** state,  $V_T$  tended to be smaller during exercise in glycogen depleted subjects, but the difference was not statistically significant. At the point of exhaustion the group mean  $V_T$  was  $2.25 \pm 0.22$  l. in the **GN** condition and  $2.01 \pm 0.14$  l. in the **GD<sub>ACUTE</sub>** condition. Because  $V_T$ , which decreased during prolonged exercise, could not have contributed to the elevation in  $\dot{V}_E$  observed during exercise in glycogen depleted subjects.

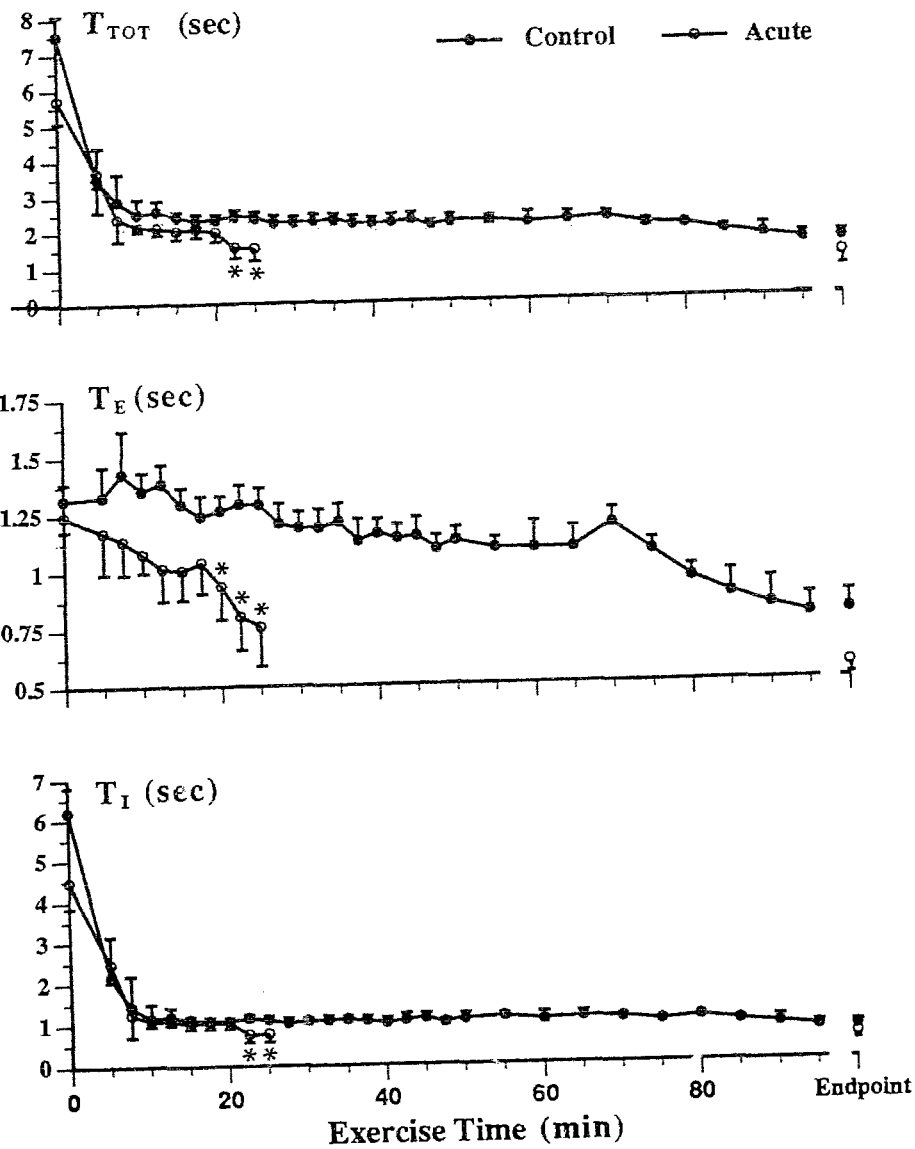
## VENTILATORY FREQUENCY

The difference in pulmonary ventilation ( $\dot{V}_E$ ) during prolonged steady state exercise between the **GN** and **GD<sub>ACUTE</sub>** state resulted from a relative increase in breathing frequency ( $f$ ) in the **GD<sub>ACUTE</sub>** condition. In the **GN** exercise test,  $f$  increased rapidly at the onset of exercise, and showed a steady upward drift after 70 minutes of prolonged exercise (Fig. 14). This corresponded with the onset of the upward drift in  $\dot{V}_E$ . Breathing frequency increased from  $8.3 \pm 0.8$  breaths $\cdot$ min $^{-1}$  at rest, to a plateau value of  $24.3 \pm 1.4$  breaths $\cdot$ min $^{-1}$  at the 10th minute of exercise, then increased to  $39.3 \pm 3$  breaths $\cdot$ min $^{-1}$  at the endpoint of exercise in the **GN** condition.

At rest, and during unloaded pedalling,  $f$  was not affected by the prior depletion of muscle glycogen. However, once the work rate increased, the difference in breathing frequency between a subject in the control and acute glycogen depleted state became greater as the duration of exercise progressed. Breathing frequency was consistently higher in the glycogen depleted subject, and significantly greater after 20 minutes of exercise. At 25 minutes of exercise,  $f$  was  $25.5 \pm 1.6$  breaths $\cdot$ min $^{-1}$  in the GN condition compared with  $45.8 \pm 9.6$  breaths $\cdot$ min $^{-1}$  in the GD<sub>ACUTE</sub> condition. The difference was greater at the endpoint of the exercise, when  $f$  was  $39.3 \pm 3.0$  Hz in the GN condition compared with  $57.2 \pm 8.9$  Hz in the GD<sub>ACUTE</sub> condition.

#### **THE DRIVE ( $V_T/T_I$ ) AND TIMING ( $T_I/T_{TOT}$ ) COMPONENTS OF VENTILATION**

In the GN condition,  $V_T/T_I$  increased rapidly at the onset of steady state exercise, then remained at a plateau until after the 80th minute of exercise when a gradual upward drift in  $V_T/T_I$  was observed (Fig. 14). Exercise in a glycogen depleted state was accompanied by a slight increase in  $V_T/T_I$  relative to the control condition, and this difference was significant in the latter phase of exercise. The pattern of the change in  $T_I/T_{TOT}$  responded as a mirror image of  $V_T/T_I$  (Fig. 14).  $T_I/T_{TOT}$  decreased with the onset of exercise, then remained essentially constant throughout exercise.  $T_I/T_{TOT}$  tended to be greater during exercise in glycogen depleted subjects, but overall this difference was not significant.



**Figure 15** Ventilatory timing components [inspired time ( $T_I$ ), expired time ( $T_E$ ), and time of one complete cycle ( $T_{TOT}$ )] in responses to prolonged cycle ergometer exercise at 50%  $WR_{max}$ . All values are mean  $\pm$  SEM. The group mean data points are expressed as in Fig. 12. \* Significantly different from GN (control) condition at that corresponding time ( $p \leq 0.05$ ).



## VENTILATORY TIMING ( $T_{TOT}$ , $T_I$ , $T_E$ )

The relationship between inspiratory timing components ( $T_{TOT}$ ,  $T_I$ ,  $T_E$ ) during prolonged steady state exercise in the **GN** and **GD<sub>ACUTE</sub>** conditions is shown in Fig. 15. Inspiratory time ( $T_I$ ) was calculated as the difference between the total time of one respiratory cycle ( $T_{TOT}$ ) and expiratory time ( $T_E$ ), each of which were measured directly.

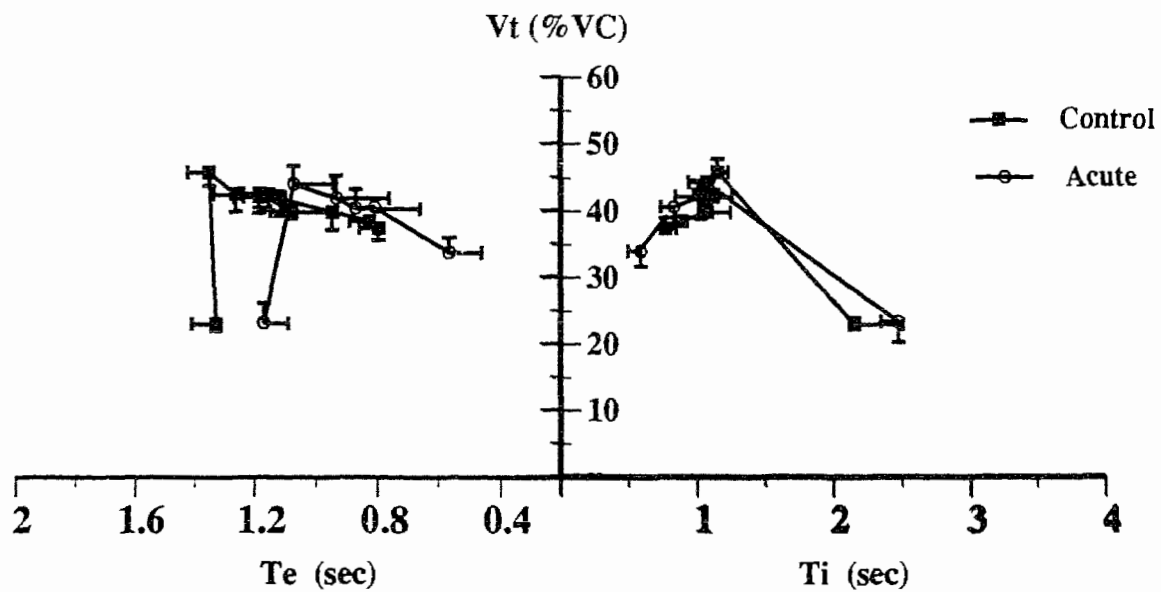
At the onset of exercise, there a rapid decrease in the total time of one breath cycle ( $T_{TOT}$ ) in both the **GN** and **GD<sub>ACUTE</sub>** condition was observed. This rapid decrease in  $T_{TOT}$  at the onset of exercise, was effected by a noticeable shortening of  $T_I$ , while  $T_E$  decreased only slightly. After the initial decrease,  $T_{TOT}$  remained constant until after the 70th minute of exercise in the **GN** condition, when there was a gradual but significant reduction in  $T_{TOT}$  by the endpoint of exercise. This reduction was caused by a decrease in  $T_E$ .  $T_{TOT}$  was significantly shorter in the **GD<sub>ACUTE</sub>** condition after the 22.5 minutes of exercise compared with the **GN** condition, which was caused by a significant reduction in  $T_E$ .  $T_I$  was not significantly different throughout exercise in either condition studied.

## THE RELATIONSHIP OF TIDAL VOLUME TO INSPIRATORY AND EXPIRATORY DURATION

The  $V_T$ - $T_I$ - $T_E$  diagram in Fig. 16 shows the group mean relationship between tidal volume (%VC) and respiratory timing ( $T_I$  and  $T_E$ ), in response to prolonged steady state exercise at 50%  $WR_{MAX}$ . At the onset of the step increase in work rate, there was a rapid increase in tidal volume and breathing frequency, resulting from a reduction in  $T_I$ , without a decrease in  $T_E$ . A steady decrease in  $V_T$  was observed as exercise continued, with a concomitant, gradual shortening of both  $T_I$  and  $T_E$ . This was reflected by an increase in breathing frequency. In response to prolonged exercise, there was no obvious breakpoint in the relation between  $V_T$  and  $T_I$ , or between  $V_T$  and  $T_E$ .

During prolonged exercise, substrate supply obviously affected the relationship between  $V_T$  and  $T_E$ , without altering the relation between  $V_T$  and  $T_I$ , since expiratory time was shorter at any given  $V_T$  during exercise in glycogen depleted subjects compared with

their exercise in a control state. Thus, during prolonged exercise in glycogen depleted state, the comparative increase in breathing frequency resulted primarily from a reduced expiratory time compared to the control condition. The reduction in expiratory time also resulted in an increased expiratory flow rate ( $V_T/T_E$ ,  $l \cdot \text{min}^{-1}$ ) at any given  $V_T$  in the GD compared with the GN condition.



**Fig. 16** The relationship between tidal volume expressed as a percentage of vital capacity ( $V_T$ , %VC) and inspiratory or expiratory duration ( $T_I$  or  $T_E$ , sec) during prolonged steady exercise at 50%  $WR_{MAX}$ . All data points represent the mean  $\pm$  SEM of both variables.

## VENTILATORY EQUIVALENTS OF OXYGEN CONSUMPTION AND CARBON DIOXIDE PRODUCTION ( $\dot{V}_E/\dot{V}O_2$ , $\dot{V}_E/\dot{V}CO_2$ )

The ventilatory equivalents of oxygen consumption and carbon dioxide production, observed during prolonged steady state exercise, are shown in Fig. 17. The group mean ventilatory equivalent for oxygen consumption ( $\dot{V}_E/\dot{V}O_2$ ) was relatively constant throughout steady state exercise in the **GN** condition, and began to drift upwards after the 70th minute of exercise. In the **GD<sub>ACUTE</sub>** condition, the group mean  $\dot{V}_E/\dot{V}O_2$  tended to be higher than in the **GN** condition at the onset of the step increase in work rate, but the difference was not significant.

The group mean  $\dot{V}_E/\dot{V}CO_2$  was greater during exercise in the **GD<sub>ACUTE</sub>** condition compared with the **GN** state. This difference was also greater in magnitude than the difference in the ventilatory equivalent for oxygen observed in the two conditions. The difference in the  $\dot{V}_E/\dot{V}CO_2$  between the **GN** or **GD<sub>ACUTE</sub>** condition however, was not statistically significant. An upward drift in the group mean  $\dot{V}_E/\dot{V}CO_2$  was also observed after the 70th minute of prolonged exercise in the **GN** state, which corresponded with the upward drift in  $\dot{V}_E$ .

## END TIDAL $PO_2$ AND $PCO_2$

A comparison of the  $P_{ET}O_2$  and  $P_{ET}CO_2$  response to prolonged steady state exercise is shown in Fig. 17. The mean  $P_{ET}O_2$  decreased at the onset of exercise, and attained a relative plateau as exercise continued. In the **GN** condition, the mean  $P_{ET}O_2$  tended to be lower and more constant at about 105 Torr, compared with the gradual upward drift in the mean  $P_{ET}O_2$  from the onset of exercise in the **GD<sub>ACUTE</sub>** condition. An upward drift was also observed in the group mean  $P_{ET}O_2$  in the **GN** condition, but only after the 70th minute of exercise, at the same point that a similar pattern was observed with  $\dot{V}_E/\dot{V}O_2$ . Throughout exercise in the glycogen depleted state a steady increase in the mean  $P_{ET}O_2$  was

observed, but the difference compared with the **GN** condition was not significant. In the **GD<sub>ACUTE</sub>** condition, an upward drift in  $P_{ET}O_2$  to 118 Torr by the endpoint of exercise was observed.

The group mean  $P_{ET}CO_2$  increased rapidly from rest at the onset of unloaded pedalling and in the first few minutes after the onset of the step increase in work rate, but after the first few minutes of steady state exercise, the mean  $P_{ET}CO_2$  gradually declined as exercise continued. The group mean  $P_{ET}CO_2$  was higher during exercise in the **GN** condition than in **GD<sub>ACUTE</sub>** condition, but this difference was not significant.

### HEART RATE

A comparison of the group mean heart rate response to steady state exercise in a normal and glycogen depleted state is shown in Fig. 18. Heart rate was significantly higher at rest and throughout exercise in the **GD<sub>ACUTE</sub>** condition compared with the **GN** condition. At rest the mean HR was  $73 \pm 4$  bpm in the **GN** condition compared with  $89 \pm 6$  bpm in **GD<sub>ACUTE</sub>** condition. Once exercise began, there was an initial rapid increase in HR, followed by a slow upward drift in HR in both the **GN** and **GD<sub>ACUTE</sub>** condition. At the endpoint of exercise, the group mean HR was  $173 \pm 6$  bpm in the **GN** condition compared with  $182 \pm 3$  bpm in the **GD<sub>ACUTE</sub>** state.

### CORE TEMPERATURE

Core temperature ( $T_c$ ) increased slowly and steadily during prolonged steady state exercise (Fig. 18). The glycogen depleted condition did not significantly affect the initial core temperature, which was  $36.8 \pm 0.1^\circ C$  in the **GN** and  $36.7 \pm 0.1^\circ C$  in **GD<sub>ACUTE</sub>** state. When the change in core temperature during exercise was expressed as the  $\Delta T_c$ , or the difference between core temperature during exercise and core temperature at rest, prolonged steady state exercise induced a mean elevation in body temperature of  $1.84 \pm 0.14^\circ C$  in the **GN** condition, compared with a  $1.38 \pm 0.23^\circ C$  rise in the **GD<sub>ACUTE</sub>** state. At exercise endpoint the group mean  $T_c$  was  $38.6 \pm 0.2^\circ C$  in **GN** subjects compared with

$38.1 \pm 0.2$  °C in **GD<sub>ACUTE</sub>** subjects. In the **GN** condition of course, the exercise duration was significantly longer than for the **GD<sub>ACUTE</sub>** state.

### **RATING OF PERCEIVED EXERTION**

The rating of perceived exertion (RPE) increased throughout the prolonged exercise test (Fig. 18). Beginning with the onset of unloaded pedalling, there was a significantly greater RPE at any equivalent time point during steady state exercise in the **GD<sub>ACUTE</sub>** condition compared with **GN**. In the **GN** condition, there was a noticeable increase in RPE after the 65th minute of exercise. While the variability in the response between subjects increased as exercise progressed, evidenced by the larger standard error, overall the difference in the mean RPE between the **GN** and the **GD<sub>ACUTE</sub>** condition was significant throughout prolonged steady state exercise. At the endpoint of prolonged steady state exercise when RPE was  $7.8 \pm 1.2$  in the **GN** compared with  $9.6 \pm 0.2$  in **GD<sub>ACUTE</sub>** state.

### **HEMATOCRIT**

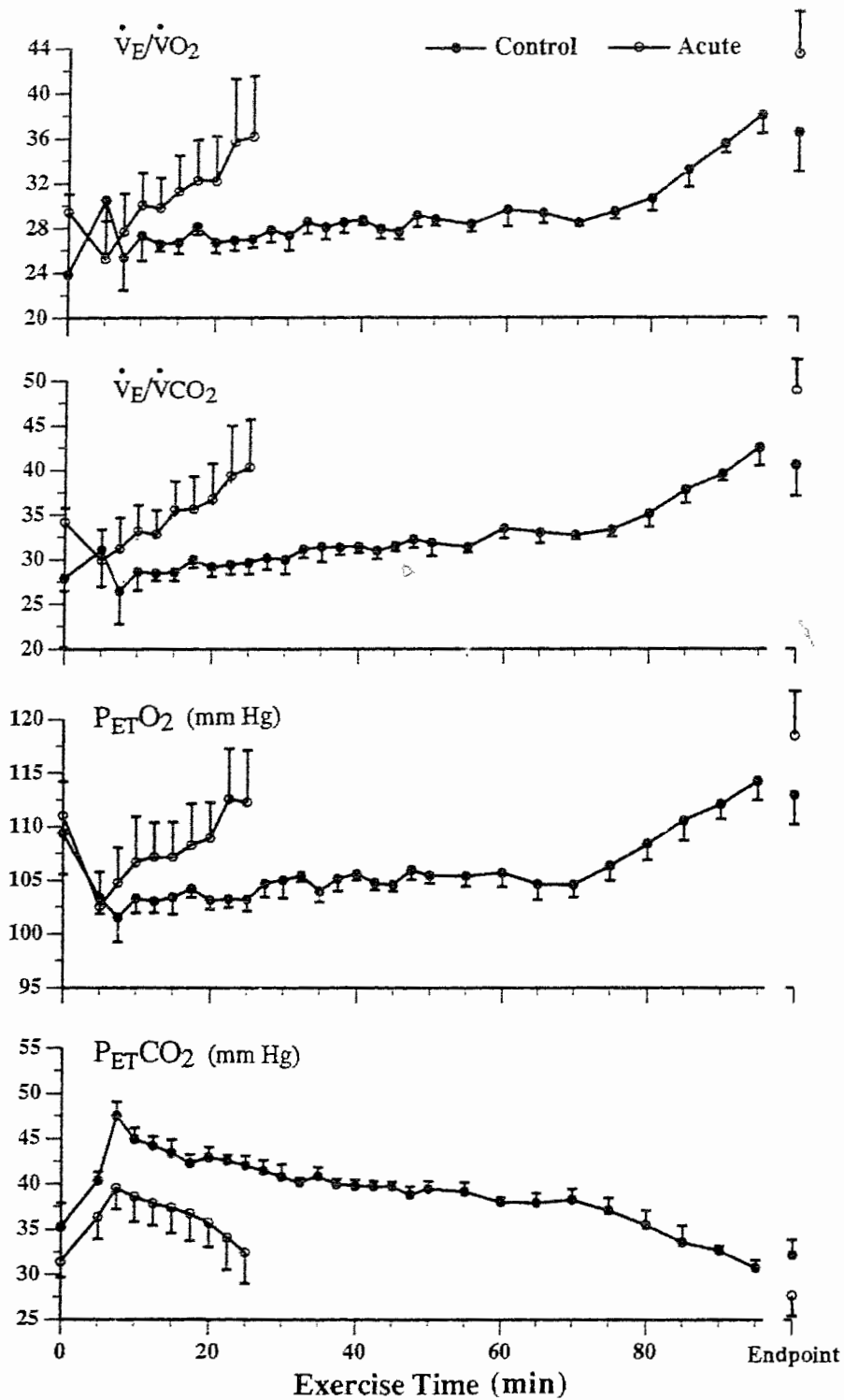
The mean hematocrit (Hct) increased significantly compared with the Hct value at rest as a result of prolonged steady state exercise in both the **GN** and the **GD<sub>ACUTE</sub>** condition (Fig. 19). There was a trend towards a lower hematocrit at equivalent absolute work rates in the **GD<sub>ACUTE</sub>** compared with the **GN** group at all times, but the difference only bordered on significance ( $p < 0.06$ ). The group mean Hct increased from a resting value of  $0.45 \pm 0.01$  to  $0.49 \pm 0.01$  at the endpoint of exercise in the **GN** condition, and from  $0.43 \pm 0.02$  to  $0.47 \pm 0.02$  at exhaustion in the **GD<sub>ACUTE</sub>** conditions.

## HEMOGLOBIN

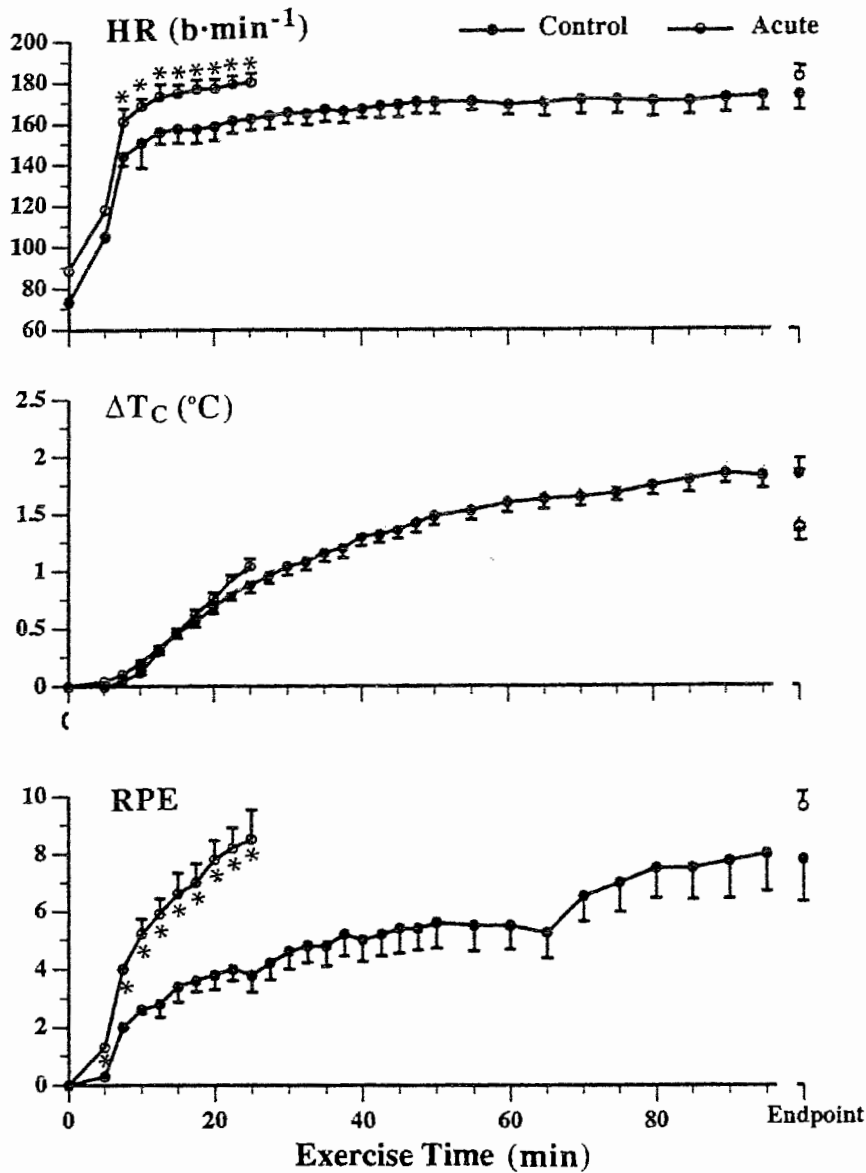
In both the **GN** and the **GD<sub>ACUTE</sub>** groups, prolonged exercise produced a significant increase in the mean hemoglobin concentration compared with the resting value. At rest the group mean Hgb was  $151.4 \pm 6.0 \text{ g}\cdot\text{l}^{-1}$  in the **GN** condition, and  $145.4 \pm 5.8 \text{ g}\cdot\text{l}^{-1}$  in the **GD<sub>ACUTE</sub>** condition, compared with  $164.8 \pm 6.2 \text{ g}\cdot\text{l}^{-1}$  and  $161.8 \pm 5.3 \text{ g}\cdot\text{l}^{-1}$  at their respective endpoints. However there was no significant difference observed between the **GN** and **GD<sub>ACUTE</sub>** conditions.

## PLASMA VOLUME

As a result of prolonged exercise, plasma volume decreased from rest to the endpoint of exercise, from  $55.3 \pm 1.4$  to  $46.6 \pm 1.5$  in the **GN** condition, and from  $56.4 \pm 0.9$  to  $49.4 \pm 1.6$  in the **GD<sub>ACUTE</sub>** state, respectively. This represented a net decrease in the group mean plasma volume of  $15.8 \pm 2.0 \%$  and  $12.3 \pm 3.1\%$  in the **GN** and **GD<sub>ACUTE</sub>** states respectively, from rest to the endpoint of prolonged exercise (Fig. 19). The decrease was significantly different from rest, but no significant difference was observed when the decrease in the mean plasma volume in the **GN** and **GD<sub>ACUTE</sub>** condition were compared.

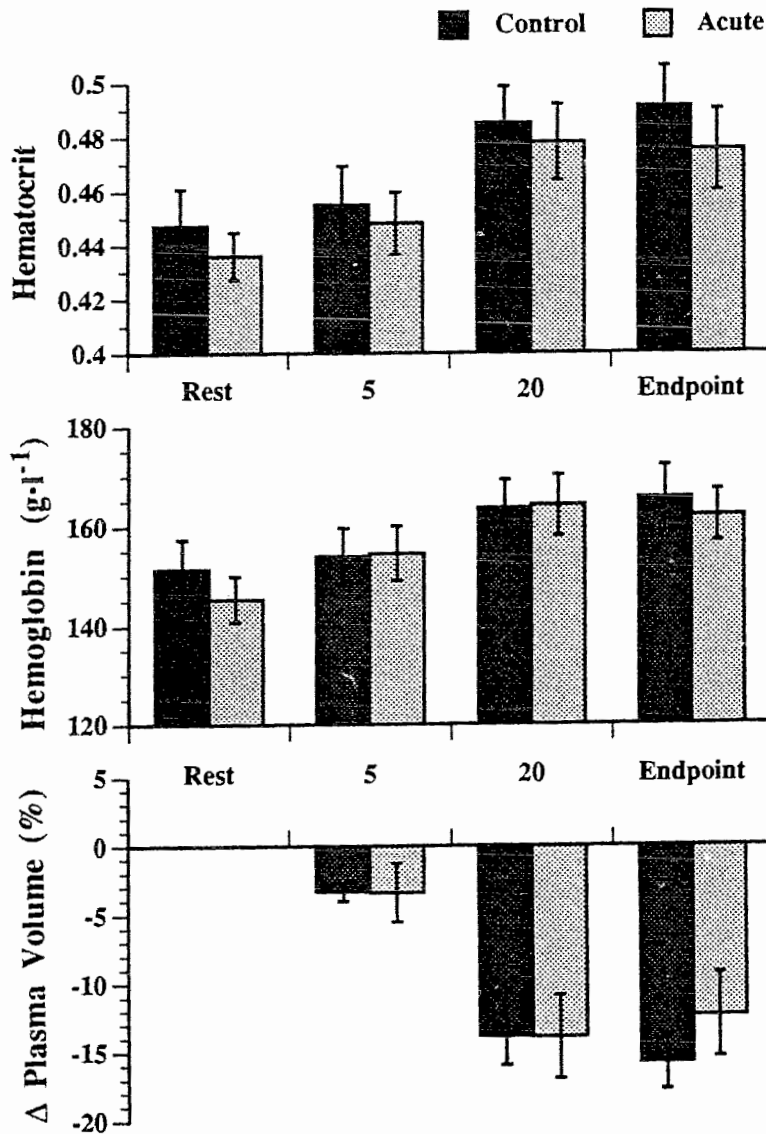


**Figure 17** Ventilatory equivalents for oxygen uptake and carbon dioxide production ( $\dot{V}_E/\dot{V}O_2$ ,  $\dot{V}_E/\dot{V}CO_2$ ) and the end-tidal  $PO_2$  and  $PCO_2$  ( $P_{ET}O_2$ ,  $P_{ET}CO_2$ ) in response to prolonged cycle ergometer exercise at 50%  $WR_{max}$ . All values are mean  $\pm$  SEM. The group mean data points are expressed as in Fig. 12.



**Figure 18** The heart rate (HR), rectal temperature ( $\Delta T_c$ ), and the rating of perceived exertion (RPE) in response to prolonged cycle ergometer exercise at 50%  $WR_{max}$ . All values are mean  $\pm$  SEM. The group mean data points are expressed as in Fig. 12.  
 \* Significantly different from GN (control) condition at that corresponding time ( $p \leq 0.05$ ).





**Figure 19** Hematocrit (Hct), hemoglobin (Hgb), and change in plasma volume ( $\Delta$ PV) in response to prolonged cycle ergometer exercise at 50%  $WR_{max}$ . Values are means  $\pm$  SEM for 5 subjects.

## BLOOD METABOLITES

A comparison of the group mean response of venous potassium, lactate, ammonia, glucose, and pH in response to prolonged steady state exercise in the **GN** and **GD<sub>ACUTE</sub>** condition are shown in Fig. 20. In the text, the blood metabolites have been expressed both as the absolute value measured, and as the concentration corrected for any plasma volume shift during exercise. Correction for the marked hemoconcentration and decrease in plasma volume observed during prolonged exercise intensity results in an effective dilution of the corrected metabolite concentration.

### AMMONIA

The response of venous ammonia ( $\text{NH}_3$ ) to exercise was significantly affected by glycogen depletion (Fig. 20). The group mean  $\text{NH}_3$  concentration was lower at rest in the **GD<sub>ACUTE</sub>** state ( $32 \pm 2 \mu\text{M}$ ) compared with the **GN** state ( $52 \pm 9 \mu\text{M}$ ), but the difference was not significant. After the initial rise at the onset of exercise, the mean  $\text{NH}_3$  concentration of **GN** was relatively stable between 20 and 60 minutes.  $\text{NH}_3$  increased from  $105 \pm 12 \mu\text{M}$  at 60 min to  $146 \pm 25 \mu\text{M}$  at the endpoint of exercise in the **GN** condition.

In the **GD<sub>ACUTE</sub>** condition,  $\text{NH}_3$  concentration increased more rapidly at the onset of prolonged steady state exercise compared with the **GN** condition, and a significantly greater increase in the group mean  $\text{NH}_3$  concentration was observed after 20 minutes of exercise compared with the observed response in the **GN** state. The final concentration of  $\text{NH}_3$  was  $146 \pm 25 \mu\text{M}$  following exercise in the **GN** state compared with  $171 \pm 15 \mu\text{M}$  in the **GD<sub>ACUTE</sub>** state, which was a significant increase from their respective resting concentration. Because of the upward drift in ammonia in the **GN** state, the concentration of ammonia was not significantly different at the endpoint of exercise. Correction of the ammonia concentration for the change in plasma volume did not affect the level of significance between the control and the glycogen depleted state.

## POTASSIUM

Prolonged steady state exercise induced a significant elevation in venous potassium concentration ( $[K^+]$ ) in both the **GN** and **GD<sub>ACUTE</sub>** condition (Fig. 20). The mean resting concentration of  $K^+$  was  $4.2 \pm 0.1$  mM and  $4.4 \pm 0.2$  mM, in the **GN** and **GD<sub>ACUTE</sub>** states respectively, which increased immediately at the onset of exercise. At the endpoint of prolonged exercise in the two conditions, the group mean venous  $[K^+]$  was  $5.1 \pm 0.1$  mM in the **GN** and  $5.2 \pm 0.1$  mM in the **GD<sub>ACUTE</sub>** state respectively. This was significantly greater than the respective resting concentration. Throughout exercise,  $[K^+]$  tended to be higher in the **GD<sub>ACUTE</sub>** condition compared with the control metabolic state. This difference however, between the two conditions was not statistically significant. When the  $[K^+]$  was corrected for a plasma volume shift with prolonged exercise, the difference between the two conditions remained non-significant.

## LACTATE

The increase in venous lactate concentration  $[La]$  was also significantly altered by prior glycogen depletion of the working muscles in response to steady state exercise, as shown in Fig. 20. At rest and during the early stage of exercise, the mean plasma  $[La]$  in the **GN** and **GD<sub>ACUTE</sub>** state was not significantly different. From the 15th minute of exercise, there was a clear separation between the venous lactate response to the prolonged exercise stimulus in the **GN** compared with the **GD<sub>ACUTE</sub>** state. In the control metabolic state, the group mean  $[La]$  reached a peak value of  $5.0 \pm 0.8$  mM at the 22nd minute of exercise, after which time it gradually decreased to  $3.6 \pm 0.3$  mM at the endpoint of exercise. Under glycogen depleted conditions, a smaller increase in lactate was observed in the early stage of prolonged exercise to a peak concentration of  $3.3 \pm 0.3$  mM at the 12th minute of exercise. After this, the  $[La]$  decreased to a mean value of  $2.9 \pm 0.2$  mM at the point of subjective exhaustion of the subject. The difference in  $[La]$  between the **GN** and **GD<sub>ACUTE</sub>** condition was significant after the 15th minute of exercise, excluding the endpoint of exercise. Correction of the venous lactate concentration for the reduction in

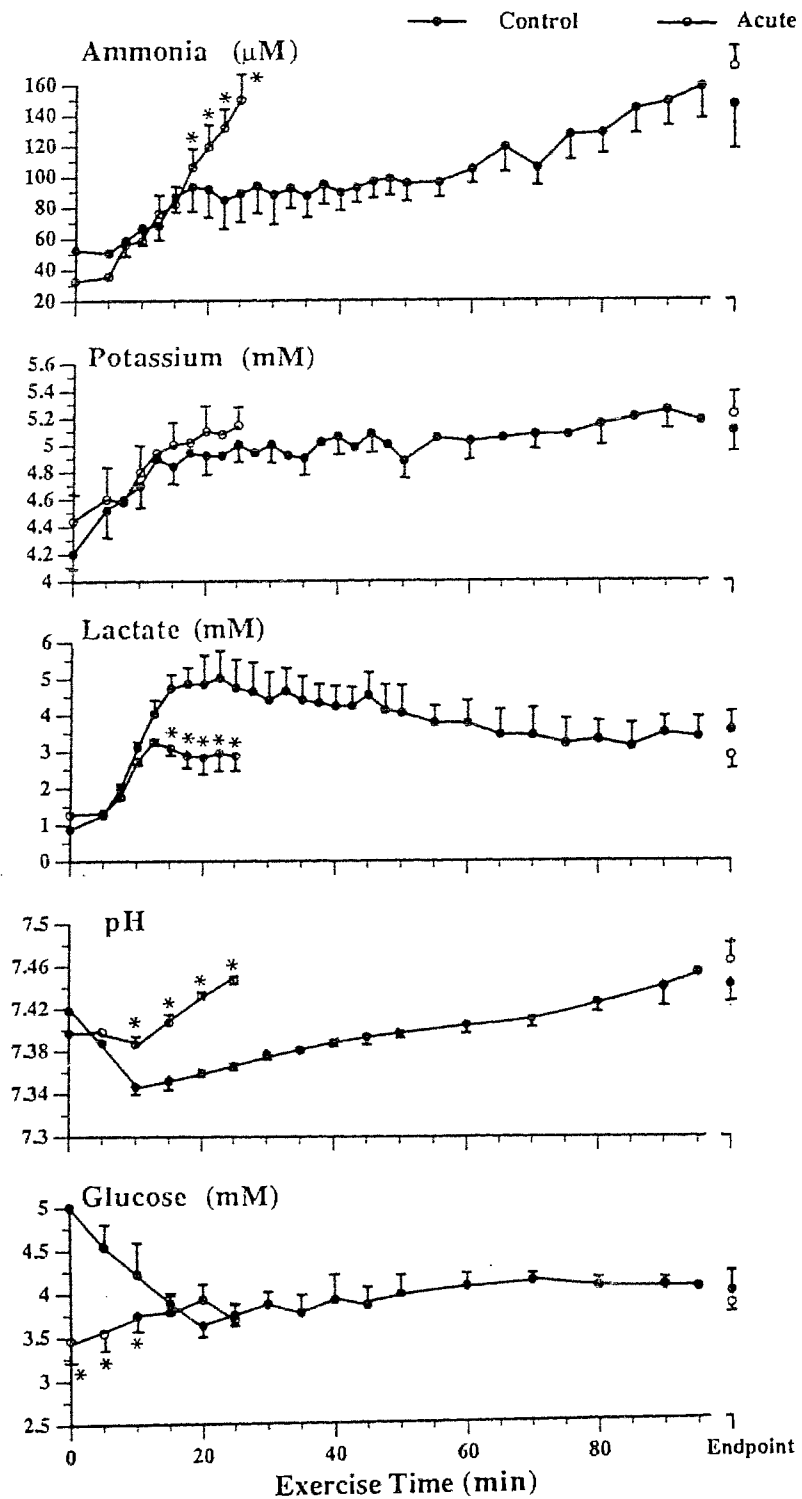
plasma volume with exercise did not change the level of significance for differences between the two groups.

## **pH**

At rest the group mean venous pH value was similar in the two metabolic conditions studied, but throughout the prolonged steady state exercise test, the mean pH value was significantly higher in the **GD<sub>ACUTE</sub>** condition (Fig. 20). At the 25th minute of prolonged exercise, a time which all subjects were able to complete in both the control and glycogen depleted protocol, the mean pH was significantly lower in the **GN** ( $7.37 \pm 0.004$ ) compared with the **GD<sub>ACUTE</sub>** ( $7.45 \pm 0.02$ ) state. In the control condition, pH reached a nadir by the tenth minute of exercise and gradually increased as exercise continued. At the endpoint of prolonged exercise the pH was not different between the two states of initial intramuscular glycogen. At this time, the group mean pH was  $7.44 \pm 0.02$  in the **GN** compared with  $7.46 \pm 0.02$  in the **GD<sub>ACUTE</sub>** state. pH corrected to the subject's core temperature, concomitant with the blood sample, was also significantly higher in the glycogen depleted state. The similarity in pH at the endpoint may be accounted for by the difference in duration of exercise in the glycogen depleted state compared with the control.

## **GLUCOSE**

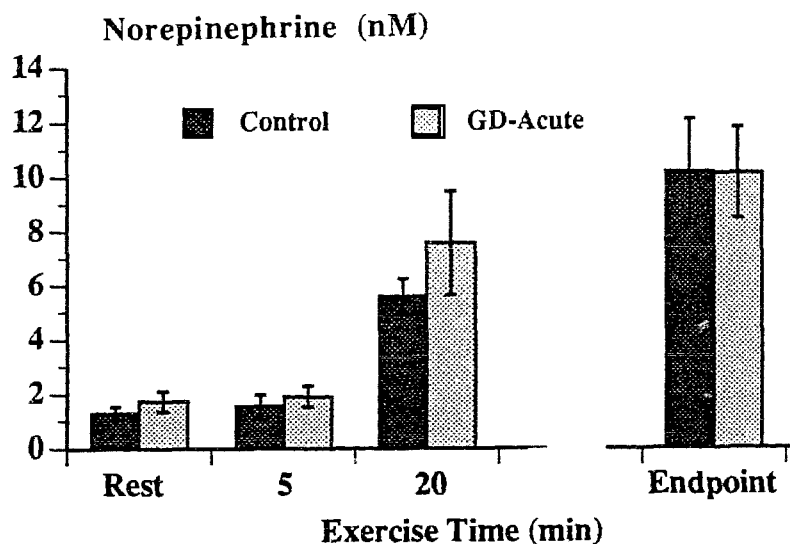
At rest, the group mean blood glucose concentration was significantly lower in the **GD<sub>ACUTE</sub>** subjects compared with the **GN** condition; ( $5.0 \pm 0.3$  mM and  $3.5 \pm 0.4$  mM in **GN** and **GD<sub>ACUTE</sub>** respectively). Once exercise began, the group mean blood glucose concentration decreased more rapidly in the control than in the glycogen depleted state. After the 10th minute of exercise, the difference in blood glucose was not significant between the two groups. At the endpoint of exercise, the group mean blood glucose concentration was  $3.94 \pm 0.19$  mM in the **GN** condition, compared with  $3.80 \pm 0.51$  in the **GD<sub>ACUTE</sub>** condition (Fig. 20). This difference was not significant.



**Figure 20** Blood metabolite changes (ammonia, [NH<sub>3</sub>]; potassium, [K<sup>+</sup>]; lactate, [La]; pH; Glucose) in response to prolonged cycle ergometer exercise at 50% WR<sub>max</sub>. All values are mean ± SEM. The group mean data points are expressed as in Fig. 12.  
 \* Significantly different from GN (control) condition at that corresponding time (p < 0.05).

## NOREPINEPHRINE

Plasma norepinephrine concentration was determined at rest, at the end of unloaded pedalling (5 min), at 20 minutes, and at the endpoint of each exercise session. In the **GN** condition, a sample was obtained from each subject at all time points. In the **GD<sub>ACUTE</sub>** condition however, the catecholamine values at 30 and 40 minutes represent data from only one subject, and were therefore not included in the statistical analysis. Plasma norepinephrine increased significantly in response to prolonged steady state exercise from its resting concentration of  $1.3 \pm 0.2$  nM and  $1.7 \pm 0.3$  nM in the **GN** and the **GD<sub>ACUTE</sub>** conditions, respectively, to  $10.1 \pm 1.9$  nM and  $10.1 \pm 1.7$  nM at the endpoint of exercise in the **GN** and the **GD<sub>ACUTE</sub>** condition, as shown in Fig. 21. The difference in plasma norepinephrine concentration between the glycogen depleted and the the **GN** condition was not significant.



**Figure 21** Plasma norepinephrine in response to prolonged cycle ergometer exercise at 50%  $WR_{max}$ . Values are means  $\pm$  SEM for 5 subjects. \* Significantly different from **GN** (control) condition at that corresponding time ( $p \leq 0.05$ ).

## RELATIONSHIP BETWEEN VENTILATION AND MEDIATORS OF VENTILATION DURING PROLONGED EXERCISE AT A CONSTANT WORK RATE

The response of ventilation ( $\dot{V}_E$ ) and breathing frequency to prolonged constant work rate exercise is compared with several potential mediators of the ventilatory response in Fig. 22. The group mean response of venous ammonia, potassium, pH, norepinephrine, and core temperature ( $\Delta T_C$ ), which individually have been proposed to stimulate ventilation, were compared. To visualize the relationship between the change in ventilation and each potential mediator in response to prolonged, constant work rate exercise, individual scattergrams are shown in Appendix IV, Fig. 39-48.

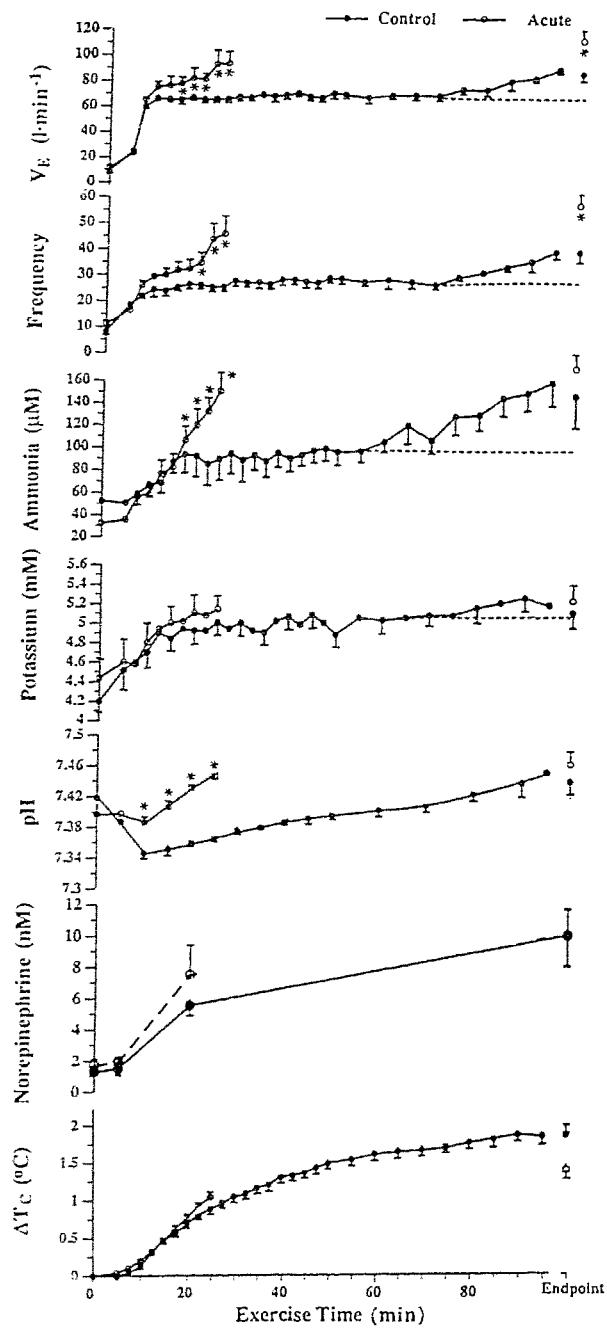
Ventilation showed a gradual upward drift after 70 min of prolonged exercise in the GN condition and tended to be higher from the onset of exercise in the glycogen depleted subject. This difference was significant from 17.5 min onwards. The rapid increase in ventilation in the first 5 minutes of the step increase in work at the onset of exercise was not matched by an equivalent increase in any mediator of ventilation measured in this study. Neither venous ammonia nor potassium, which increased from the onset of exercise, had a time course that was sufficiently fast to account for the sudden increase in ventilation at the onset of exercise. The change in venous pH, which decreased at the onset of exercise, and was significantly higher in the glycogen depleted subject, also could not match the observed increase in ventilation at the onset of exercise. The slow increase in core temperature increased at the onset of the work rate did not match the initial rapid increase in ventilation.

In the control, GN condition, ventilation remained relatively constant at approximately 65 litres from the 10th to the 70th minute of exercise. During this time,  $[\text{NH}_3]$  increased from 90 to 110  $\mu\text{M}$ ,  $[\text{K}^+]$  remained constant at 5.0 mM, pH increased from 7.34 to 7.40, and temperature rose by 1.1  $^\circ\text{C}$ . A noticeable upward drift in ventilation was observed in the last 20 minutes of prolonged exercise in the control condition, to  $86.7 \pm 5.1 \cdot \text{min}^{-1}$ , an increase of >20 litres from the 70th minute of exercise. The positive drift in ventilation was caused by an increase in breathing frequency. Over the

specific period of time when ventilation showed a positive drift in the control condition,  $[\text{NH}_3]$  increased from 110 to 146  $\mu\text{M}$ ,  $[\text{K}^+]$  increased by 0.1 mM to 5.1 mM, pH continued to increase to an Endpoint value of 7.44, and  $\Delta T_C$  increased by a further 0.2  $^\circ\text{C}$ . Norepinephrine increased in response to constant work rate exercise, but was only measured at 5 min, 20 min, and at the Endpoint of exercise. Thus a direct comparison between it, and the pattern of change in ventilation, could not be made.

The initial increase in ventilation in the glycogen depleted condition, which tended to be greater from the onset of exercise compared with the control state, could not be accounted for by an obvious increase in any of the mediators of ventilation relative to the control value measured in this study. Up to the 17.5 minute of exercise, the concentration of ammonia and potassium, and the change in core temperature, were not significantly different in the glycogen depleted compared with the control condition. Although pH was significantly higher in the glycogen depleted group, this relative alkalosis is opposite to the change in  $[\text{H}^+]$  expected to stimulate ventilation. However during exercise in the glycogen depleted condition, ammonia concentration continued to rise, and was significantly greater from the 20 minute of exercise compared with the control condition. At this time, ventilation continued to increase, with a concomitant increase in ventilatory frequency. During prolonged exercise in the glycogen depleted condition, neither potassium nor core temperature were significantly greater compared with the control state.





**Fig. 22** Effects of prolonged constant work rate exercise at 50% WR<sub>MAX</sub> on minute ventilation ( $V_E$ ), breathing frequency, venous ammonia, potassium, pH, norepinephrine and change in core temperature ( $\Delta T_c$ ). All values are means + SEM. \* indicates significantly different from the GN (control) condition.

## CORRELATION OF RATING OF PERCEIVED EXERTION WITH PHYSIOLOGICAL AND BIOCHEMICAL MEASURES PROLONGED EXERCISE

During prolonged exercise at a constant work rate of 50%  $WR_{MAX}$  for each subject, the group mean subjective rating of perceived exertion increased with exercise duration (Fig. 18) RPE was also significantly higher during exercise in a glycogen depleted state. Table 6 shows that a subject's RPE measured from the onset of the step increase in work rate to the Endpoint in the GN condition correlates most strongly with  $\Delta[NE]$ ,  $\Delta[NH_3]$ ,  $\Delta pH$ ,  $\Delta[K^+]$ ,  $\Delta T_c$ , and  $\Delta \dot{V}_E$ . Unexpectedly, however, the correlation of RPE with  $\Delta pH$  is positive. During the latter portion of exercise, from 60 minutes to the Endpoint, RPE correlates most strongly with  $\Delta \dot{V}_E$ ,  $\Delta[NH_3]$ , and a fall in blood glucose concentration. During exercise in the  $GD_{ACUTE}$  state, RPE is most strongly correlated with  $\Delta[NH_3]$  and  $\Delta \dot{V}_E$ .

**Table 6** The Pearson correlation coefficient of the change in RPE ( $\Delta RPE$ ) versus  $\Delta \dot{V}_E$ ,  $\Delta[NH_3]$ ,  $\Delta[K^+]$ ,  $\Delta pH$ ,  $\Delta[La]$ ,  $\Delta HR$ ,  $\Delta[NE]$ ,  $\Delta T_c$ , and  $\Delta$  Glucose in prolonged steady state exercise at 50%  $WR_{max}$ . The delta ( $\Delta$ ) values were calculated as the difference from rest in each variable at a specific time point. The number in parentheses indicates the (n) for each correlation coefficient.

Condition	$\Delta \dot{V}_E$	$\Delta[NH_3]$	$\Delta[K^+]$	$\Delta pH$	$\Delta[La]$	$\Delta HR$	$\Delta[NE]$	$\Delta T_c$	$\Delta$ Glucose
GN (overall)	0.62 (136)	0.71 (136)	0.47 (136)	0.65 (74)	0.28 (136)	0.46 (136)	0.74 (25)	0.63 (136)	-0.56 (74)
GN: (60 min → Endpoint)	0.50 (28)	0.49 (28)	0.04 (28)	0.25 (18)	0.27 (28)	-0.19 (28)		0.02 (28)	-0.67 (18)
$GD_{ACUTE}$	0.69 (67)	0.85 (67)	0.60 (67)	0.50 (39)	0.60 (67)	0.57 (67)	0.62 (17)	0.48 (67)	-0.37 (39)

## SUMMARY: RESULTS OF PROLONGED EXERCISE

Prolonged steady state exercise at 50%  $WR_{MAX}$  increased  $\dot{V}_E$ ,  $\dot{V}O_2$ , and  $\dot{V}CO_2$  at the onset of the work rate. In the early stage of exercise in the control glycogen condition,  $\dot{V}_E$ ,  $\dot{V}O_2$ , and  $\dot{V}CO_2$  were relatively constant. However as exercise progressed, a gradual upward drift in  $\dot{V}_E$  was observed.  $\dot{V}_E$  was significantly greater during prolonged steady state exercise in the  $GD_{ACUTE}$  condition compared with the control,  $GN$  state, although the total exercise time tolerated by an individual was significantly reduced.

The ventilatory drift observed during prolonged exercise was associated with a gradual decrease in tidal volume, but a higher ventilatory frequency. A gradual upward drift in  $V_T/T_I$ , assumed to reflect ventilatory drive, was observed during prolonged exercise in the  $GN$  state, and was higher during exercise in the glycogen depleted state compared with the control condition. Analysis of the  $V_T$ - $T_I$ - $T_E$  diagram showed that during prolonged exercise in a glycogen depleted state, expiratory time was shorter at any given  $V_T$  compared with a control state. Thus, expiratory flow rate ( $V_T/T_E$ ,  $l \cdot \text{min}^{-1}$ ) was increased in the  $GD$  compared with the  $GN$  condition, without a significant change in inspiratory flow rate ( $V_T/T_I$ ,  $l \cdot \text{min}^{-1}$ ).

During prolonged exercise in the control,  $GN$ , condition, venous ammonia concentration first increased at the onset of exercise, then showed a later, secondary rise as exercise progressed. Venous potassium concentration also increased at the onset of exercise, but then remained constant for the remainder of exercise. pH, which decreased slightly at the onset of exercise, gradually and progressively drifted upward, so that by the end of exercise, a relative alkalosis was observed. Exercise in the glycogen depleted state resulted in a greater increase in venous ammonia concentration, no difference in venous potassium, and a reduction in venous lactate accumulation compared with exercise in the control condition. In the glycogen depleted state, pH also tended to increase from the onset of exercise. Norepinephrine increased significantly in both the control and the glycogen

depleted conditions. Core temperature, which increased gradually and continuously throughout prolonged exercise, was not significantly different at any given time point when a subject was glycogen depleted.

In response to prolonged exercise in the control exercise condition, the pattern of change in ventilation was compared with  $[\text{NH}_3]$ ,  $[\text{K}^+]$ ,  $[\text{NE}]$ , and  $T_c$ . Because pH increased throughout prolonged exercise, which is opposite to what might be expected if  $\text{H}^+$  played a significant role in the stimulation of ventilation, it was discounted as a ventilatory mediator under these conditions. The perception of effort (RPE) throughout prolonged exercise was significantly greater at any given time point when a subject was glycogen depleted. RPE was most strongly correlated with  $\Delta\dot{V}_E$ ,  $\Delta[\text{NH}_3]$ ,  $\Delta[\text{NE}]$ , and  $\Delta T_c$ .

## DISCUSSION

### MUSCLE GLYCOGEN

Although glycogen concentration from biopsied muscle was not analyzed in the present study, several factors suggest that muscle glycogen was significantly reduced in the  $GD_{ACUTE}$  and  $GD_{CHRONIC}$  condition, respectively.

Each subject completed a strenuous glycogen depletion protocol which involved prolonged steady state exercise, followed by repeated supramaximal sprint intervals. This was followed by a ketogenic diet containing a reduced carbohydrate content and a high proportion of protein and fat (Table 4). A similar exercise and diet protocol has been validated previously by analysis of a muscle biopsy to produce low intramuscular glycogen (Bergstrom, *et al.*, 1967; Gollnick, *et al.*, 1973; Gollnick, *et al.*, 1974; Heigenhauser, *et al.*, 1983; Vollestad and Blom, 1985).

At every work rate in both prolonged and ramp exercise, the R value was significantly reduced in the glycogen depleted state, compared with the control (Fig. 1,12). A reduced R value reflects a shift in substrate utilization towards a greater reliance on fat and a reduction in the proportion of carbohydrate metabolized (Jansson, 1980).

A changed substrate selection and carbohydrate supply were also reflected in the reduced group mean blood lactate concentration during exercise in a glycogen depleted state (Fig. 9, 20). This observation has been reported in numerous other studies (Karlsson and Saltin, 1971; Jansson, 1980; Jacobs, 1981b; Hughes, *et al.*, 1982; Hargreaves, *et al.*, 1984).

Glycogen depletion was accompanied in each subject by a significantly reduced blood glucose concentration both at rest and in the early stages of prolonged exercise, and throughout ramp exercise (Fig. 9, 20). These observations are similar to previous observations (Jansson, 1980). Some studies, however, have reported no significant

difference, at least in resting blood glucose, in different states of glycogen depletion and dietary manipulation (Kirwan, *et al.*, 1990).

Glycogen depleted subjects fatigued sooner in both the incremental and the prolonged steady state exercise than in the control metabolic state in the present study (Table 5, 15). It has been well established previously that depleted intramuscular glycogen is correlated with reduced exercise performance (Karlsson and Saltin, 1971; Jacobs, 1981a; Hughes, *et al.*, 1982; Hargreaves, *et al.*, 1984).

### VENTILATION AND BREATHING PATTERN

In the present study, pulmonary ventilation ( $\dot{V}_E$ ) was significantly increased by glycogen depletion in response to prolonged exercise, and during intense ramp incremental exercise. These results agree with the previous studies by Hughes, *et al.*, (1982) and Heigenhauser, *et al.*, (1983), Segal and Brooks (1979) and Green, *et al.*, (1979). However, Podolin, *et al.*, (1991), reported a decrease, and McLellan and Gass (1989b) showed no change in  $\dot{V}_E$  in response to exercise in the glycogen depleted, compared with the normal glycogen state.

During ramp exercise,  $\dot{V}_E$  increased in response to the incremental work rate by increasing tidal volume and breathing frequency (Fig. 3). A similar pattern of increase in  $\dot{V}_E$  was described by Hey, *et al.*, (1966), who reported that tidal volume increases linearly with an increase in  $\dot{V}_E$ , up to a tidal volume about half the vital capacity. With a further increase in  $\dot{V}_E$ ,  $V_T$  plateaued, and  $\dot{V}_E$  was augmented by an increase in the frequency of breathing. Several other studies have confirmed this change in breathing pattern in response to incremental exercise (Lind, 1984; Lind and Hesser, 1984a; Mekjavic, *et al.*, 1987; Mekjavic, *et al.*, 1991).

The present study also agrees the previous observation that prolonged steady state exercise results in a steady upward drift in pulmonary ventilation (Costill, 1970; Dempsey, *et al.*, 1977; Wasserman, 1978; Martin, *et al.*, 1981; Hanson, *et al.*, 1982). The pattern

of ventilation, however, is remarkably different from that observed during incremental exercise. The positive drift in ventilation during prolonged exercise in the control and glycogen depleted condition is associated with a reduction in  $V_T$ , and a concomitant increase in breathing frequency. This strategy to increase pulmonary  $\dot{V}_E$  is considered inefficient (Dempsey and Manohar, 1992). If the dead space to tidal volume ratio ( $V_D:V_T$ ) increases, which would occur with an increase in breathing frequency and fall in tidal volume, alveolar ventilation will be reduced, and  $PaCO_2$  will rise. In the present study,  $PaCO_2$  and  $V_D$  were not determined. However, end tidal  $PCO_2$ , which gives an indication of the change in  $PaCO_2$  in a normal healthy individual, decreased in response to the upward drift in ventilation in prolonged steady state exercise (Fig. 17). At a high metabolic rate, the alveolar-arterial difference in  $PCO_2$  is slightly negative (Cerretelli and Di Prampero, 1987). Thus the  $P_{ET}CO_2$ , measured in this study, may underestimate  $PaCO_2$  during prolonged exercise.

The increased breathing frequency observed in the present study mirrored a reduction in the time of a breathing cycle,  $T_{TOT}$  (Fig. 15). While inspiratory time ( $T_I$ ) remained relatively constant throughout prolonged exercise, a significant shortening of expiratory time ( $T_E$ ) reduced  $T_{TOT}$ , and increased  $f$ . These results during prolonged exercise are similar to those of Cunningham and Gardner (1972, 1977) who produced the same change in breathing pattern in man by a hypercapnic drive to  $\dot{V}_E$ . Kay, *et al.*, (1975) also observed that in steady state exercise the increase in breathing frequency correlated well with a steady decrease in  $T_E$ .

It has been suggested that the metabolic cost of breathing will increase if the reduction in the expiratory time is sufficient to bring the expiratory flow rate to the limit of the maximum flow:volume loop (Petersen, 1987; Sharrat, *et al.*, 1987; Henke, *et al.*, 1988; Dempsey and Manohar, 1992). Estimates which have been made of the cost of breathing during exercise suggest that 15-20% of the total  $O_2$  uptake is due to the cost of breathing in humans at a ventilation above  $100 \text{ l}\cdot\text{min}^{-1}$  (Petersen, 1987). This has been

confirmed by the measurement of diaphragmatic blood flow and arteriovenous  $O_2$  differences in the dog (Bye, *et al.*, 1983). In exercising ponies, 14-15% of the total cardiac output is diverted to respiratory muscle at maximum work rates (Manohar, 1990).

In the current study, the metabolic cost of respiratory muscle activity was not assessed. However, during prolonged exercise, the positive drift in  $\dot{V}_E$  was not accompanied by a concomitant rise in  $\dot{V}O_2$  (Fig. 12); in the incremental exercise test,  $\dot{V}O_2$  did not deviate from a linear increase associated with the ramp increment in work rate (Fig. 1). One might expect an upward deviation in  $\dot{V}O_2$  in the above conditions if the metabolic cost of ventilation is high. Without an additional increase in  $\dot{V}O_2$ , then the respiratory muscles must divert  $O_2$  from active skeletal muscle, thereby increasing their reliance on glycolysis for energy production. This may contribute to lactate and  $H^+$  production, especially during ramp exercise. However, in the present study, it is not sufficient to result in an increase in lactate or  $H^+$  accumulation in the later stage of prolonged steady state exercise, when both blood lactate and  $[H^+]$  decreased (Fig. 20).

#### DRIVE AND TIMING COMPONENTS OF $\dot{V}_E$

The present study is the first to report that glycogen depleted subjects have a significant increase in ventilatory drive ( $V_T/T_I$ ) in both prolonged steady state, and ramp incremental exercise to exhaustion, compared with equivalent exercise in a normal metabolic state (Fig. 3, 14). The derived timing component of ventilation,  $T_I/T_{TOT}$ , showed an initial drop from rest at the onset of steady state exercise, then increased progressively with exercise duration. An initial reduction in  $T_I/T_{TOT}$  was also observed at the onset of ramp incremental exercise, which plateaued as exercise intensity increased.

$V_T/T_I$  reflects the intensity of neuromuscular inspiratory drive, and  $T_I/T_{TOT}$  represents inspiratory timing (Milic-Emili, *et al.*, 1981). An increase in  $V_T/T_I$  has previously been associated with an increase in the activity of inspiratory  $\alpha$ -motoneurons during inspiration (Milic-Emili, *et al.*, 1981; Milic-Emili, 1983). Several studies have



shown that exercise increases  $V_T/T_I$  (Askanazi, *et al.*, 1979; Hesser and Lind, 1983; Lind and Hesser, 1984a).  $T_I/T_{TOT}$  has been reported to increase throughout incremental exercise (Hesser and Lind, 1983; Lind and Hesser, 1984a), or to increase up to twice control  $\dot{V}_E$ , then reach a plateau (Askanazi, *et al.*, 1979).

Environmental conditions may also affect the pattern of breathing. Martinet *et al.*, (1979) observed that an increase in body temperature of 0.8°C increased breathing frequency and reduced tidal volume at a constant  $\dot{V}_E$ , although the drive ( $V_T/T_I$ ) and timing ( $T_I/T_{TOT}$ ) components of ventilation were unaffected by this increase in core temperature. Hypoxia stimulates  $\dot{V}_E$  predominantly by stimulation of peripheral chemoreceptors (Cunningham *et al.*, 1986). Increased  $\dot{V}_E$  observed during hypoxic exercise was associated with a significant increase in  $V_T/T_I$  suggesting increased respiratory drive, but no change in  $T_I/T_{TOT}$  (Mekjavic *et al.*, 1987). Hypobaric hypoxia significantly increased both the  $V_T/T_I$  and  $T_I/T_{TOT}$  components of ventilation during exercise (Mekjavic *et al.*, 1991).

#### **THE EFFECT OF GLYCOGEN DEPLETION ON THE RELATIONSHIP BETWEEN TIDAL VOLUME AND RESPIRATORY TIMING PATTERN DURING EXERCISE**

The  $V_T$ - $T_I$ - $T_E$  diagrams show the relationship between tidal volume and respiratory timing during ramp (Fig. 5) and prolonged steady state exercise (Fig. 16). For comparison, Fig. 23 shows the pattern of the group mean  $V_T$ - $T_I$ - $T_E$  response to ramp exercise in the GN condition superimposed on the breathing pattern observed during prolonged exercise.

In glycogen depleted subjects, the pattern of the  $V_T$ - $T_I$  and  $V_T$ - $T_E$  relationship altered in response to both ramp and prolonged exercise. During ramp exercise, the pattern in the  $V_T$ - $T_I$ - $T_E$  diagram is similar in control and glycogen depleted subjects, but the curve is shifted (Fig. 5). Thus a lower tidal volume was generated for any given  $T_I$  or  $T_E$  during exercise in glycogen depleted subjects compared with controls.

During prolonged exercise, the predominant affect of glycogen depletion appears on the  $V_T$ - $T_E$  plot (Fig. 16; Fig. 23). Throughout exercise,  $T_E$  gradually decreased with only a small reduction in  $V_T$ .

The duration of inspiration and expiration are affected by the summation of central drive and volume-related factors, although the relative importance of each factor during exercise is not fully understood.  $T_I$  is shortened by negative feedback from pulmonary stretch receptors through the Hering-Breuer (H-B) reflex (Berger and Horbein, 1989), by input from the pneumotaxic centre (Cohen, 1979), and by an increase in activity of an inspiratory 'off-switch' (Euler, 1983).

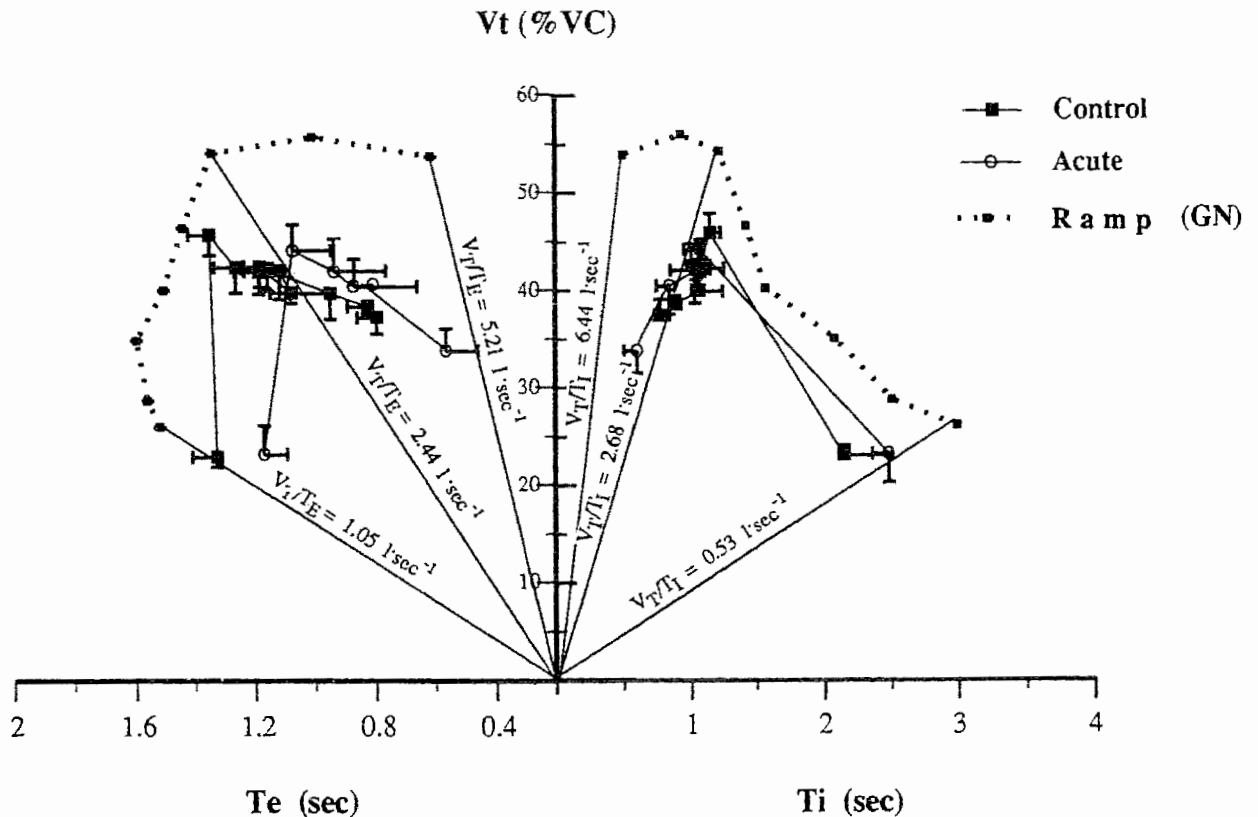


Fig. 23 The  $V_T$ - $T_I$ - $T_E$  diagram showing the relationship between tidal volume and inspiratory or expiratory duration during prolonged steady exercise at 50%  $WR_{MAX}$ . The dashed lines represent the  $V_T$ - $T_I$  and  $V_T$ - $T_E$  relationship observed during ramp incremental exercise in the GN condition. The isopleths radiating from the origin are mean inspiratory ( $V_T/T_I$ ,  $l \cdot sec^{-1}$ ) and expiratory ( $V_T/T_E$ ,  $l \cdot sec^{-1}$ ) flow rates corresponding to the group mean value at each indicated point. The flow rates were calculated at 4 min, 14 min, and at the endpoint of ramp incremental exercise.

In the Hering-Breuer reflex, first described by Hering and Breuer in 1868 and reflected in the  $V_T$ - $T_I$  diagram,  $T_I$  is the time taken before the feedback from the pulmonary stretch receptors has reached sufficient strength to inhibit inspiration. The H-B reflex, relatively weak in humans at rest, increases in activity when tidal volume increases (Berger and Hornbein, 1989). When tidal volume reaches about 50% of the vital capacity, a breakpoint is typically observed (Hey, *et al.*, 1966). A further increase in ventilation is achieved at a constant tidal volume by an increase in breathing frequency. The shift to the left in the  $V_T$ - $T_I$  relationship in response to ramp exercise is the glycogen depleted condition suggests that in this condition, the sensitivity of the HB reflex has increased.

Input from the pneumotaxic centre in the upper pons may also affect inspiratory duration. Cohen (1979) observed that discrete stimulation of the pneumotaxic centre induces a premature termination of inspiration. Furthermore, von Euler (1983) proposed that an inspiratory "off-switch" is activated when a rising centrally-generated inspiratory activity, in combination with increased afferent pulmonary stretch receptor activity, reaches a critical threshold value. Once this happens, the inspiratory motoneurons are inhibited for the duration of the subsequent expiration. An increase in central inspiratory activity (CIA) reflects the rate of rise of activity of inspiratory  $\alpha$ -motoneurons during inspiration, which in turn reflects an increase in "respiratory drive", or increased chemical or other stimulus to breathing (Milic-Emili, *et al.*, 1981; Milic-Emili, 1983).

According to the von Euler model, the pneumotaxic centre normally exerts a threshold-lowering influence on the inspiratory "off-switch" mechanism (Euler, 1983). In the present study, a lowering of the "off-switch" threshold could also explain the shift to the left of the  $V_T$ - $T_I$  curve in glycogen depleted subjects. It is possible that a change in chemical or thermal stimuli present during glycogen depleted exercise changes the sensitivity of the "off-switch" threshold. The shortening of inspiration seen in response to

a raised body temperature has previously been explained by this mechanism, possibly mediated by descending hypothalamic projections (Petersen, 1987).

The duration of expiration ( $T_E$ ) is influenced by the activation of expiratory muscles, by an increase in activity of a volume-related expiratory off-switch, and by respiratory braking (Milic-Emili, *et al.*, 1987).  $T_E$  is affected most at greater respiratory drives, when the high expiratory flows encountered are caused by active expiratory efforts of abdominal muscles (Petersen, 1987). Although less is known about the mechanisms terminating expiration, or 'triggering' the following inspiration, these may both involve a central-expiratory control which inhibits expiratory-inhibitory switching (Petersen, 1987). This activity declines with time during expiration, and eventually allowing inspiration to start.

The breakpoint observed in the  $V_T$ - $T_E$  diagram in response to ramp exercise (Fig. 5) suggests onset of increased expiratory  $\alpha$ -motoneuron activity in both control and glycogen depleted subjects. Campbell and Green (1953, 1955) have reported that in humans, significant abdominal muscles activity increases only when ventilation reaches 70-90  $l \cdot \text{min}^{-1}$ . However, the overall shift to the right in the  $V_T$ - $T_E$  diagram in response to ramp exercise in glycogen depleted subjects may reflect an increased sensitivity of an expiratory 'off-switch', or in central-expiratory control. Again, sensitivity may change with altered chemical or thermal stimuli present in the glycogen depleted state.

It has also been suggested that increased upper airway resistance during exercise may reduce a maintained diaphragmatic activity during expiration, and increase activity of the posterior cricoarytaenoid muscle (i.e. widening of the glottis) and the abdominal muscles, which together would lead to increasing expiratory flow rates (Petersen, 1987).

At any point on the  $V_T$ - $T_I$ - $T_E$  diagram, a line extrapolated through the origin reflects the isopleth of the mean rate of inspiratory and expiratory flow (Lind and Hesser, 1984b). Thus, during ramp exercise, any given mean inspiratory ( $V_T/T_I$ ) or expiratory flow ( $V_T/T_E$ ) was achieved at a smaller  $V_T$  and shorter  $T_I$  or  $T_E$ . The mean inspiratory flow reflects the

intensity of the total respiratory drive, which in turn determined the 'central inspiratory activity'; mean expiratory flow has been associated with a given recoil pattern of the lung and chest wall (Petersen, 1987).

Petersen and Vejby-Christensen (1977) observed that during short duration steady state exercise, inspiratory and expiratory time may fluctuate without influencing inspiratory or expiratory flow. Thus, on the  $V_T$ - $T_I$ - $T_E$  diagram, the mean inspiratory and expiratory flow would have a cluster of points surrounding a single isopleth of inspiratory or expiratory flow.

This is not the case in prolonged steady state exercise when a significant ventilatory drift is observed. Following the isopleths of mean inspiratory and expiratory flow in the  $V_T$ - $T_I$ - $T_E$  diagram in Fig. 23 shows that as the duration of prolonged steady state exercise increased, mean inspiratory flow rate was not affected, but the mean expiratory flow rate increased significantly. This strategy to increase pulmonary ventilation is different to the increase in both inspiratory and expiratory flow rate observed in response to ramp incremental exercise.

During prolonged exercise in the present study, several factors may account for the significant reduction of  $T_E$  during exercise in glycogen depleted subjects (Fig. 23). Either active expiration is present, and increases in activity in glycogen depleted subjects, or the threshold of the expiratory 'off-switch' is decreased. The sensitivity of central-expiratory control (which inhibits expiratory-inspiratory switching) may also have increased, leading to an earlier initiation of inspiration. The primary reason for the reduction in  $T_E$  in this study is not clear.

It is probable that at the start of  $GD_{ACUTE}$  exercise, each subject was not fully recovered from the strenuous  $GN$  exercise session, based on resting HR and blood lactate (Table 5, 15). At the onset of  $GD_{CHRONIC}$  exercise, however, it seems that the basic physiological variables used monitor recovery had returned to baseline values. In response to ramp exercise in glycogen depleted subjects, the shift in the  $V_T$ - $T_I$ - $T_E$  relationship was

similar in both glycogen depleted conditions compared with the control state (Fig. 5). Thus, while the lasting effect of prior exercise may have affected certain physiological variables at rest, the similarity between the ventilatory response observed in the  $GD_{ACUTE}$  and  $GD_{CHRONIC}$  conditions suggests that immediate prior exercise was not primarily responsible for the difference observed compared with exercise in the control condition.

#### GAS EXCHANGE ( $\dot{V}O_2$ , $\dot{V}CO_2$ , R)

In the current study, a significant increase in oxygen consumption was observed at an equivalent work rate in both ramp and prolonged exercise when subjects were glycogen depleted compared with their control condition. Carbon dioxide production, however, was not significantly different during exercise in the glycogen depleted and the control condition. The R value, an index of muscle substrate metabolism, was significantly lower in each glycogen depleted state indicating a shift towards increased fat oxidation by working muscle (Jansson, 1980).

In the present study, the difference in the group mean  $\dot{V}O_2$  between subjects in the control and both glycogen depleted conditions, gradually increased during ramp incremental exercise (Fig. 1). By the 16th minute of the ramp,  $\dot{V}O_2$  was 14 and 18% higher in the  $GD_{ACUTE}$  and  $GD_{CHRONIC}$  states respectively, compared with the control glycogen state. At the endpoint of ramp exercise,  $\dot{V}O_{2max}$  was not significantly different when the control and either glycogen depleted state of the subjects were compared, although the group mean  $WR_{max}$  at the point of exhaustion was 17% less in both glycogen depleted conditions compared with the control state. During prolonged exercise,  $\dot{V}O_2$  was consistently 4% higher when the subjects were glycogen depleted compared with the control state.

Heigenhauser, *et al.*, (1983) also reported that at any given power output,  $\dot{V}O_2$  was 5 to 20% higher during incremental exercise when muscle glycogen was reduced compared with a control state, although  $\dot{V}CO_2$  was similar in the two exercise conditions.

$\dot{V}O_{2max}$  was also similar in the above study between exercise in the control and glycogen depleted conditions, although subjects completed an average of 14% more work in the control condition. Similar data were reported by Jansson (1980), who observed a comparable elevation in  $\dot{V}O_2$  and lower R value in glycogen depleted subjects exercising at a constant work rate of 65%  $\dot{V}O_{2max}$ . Broberg and Sahlin (1988) reported a smaller, but significant increase of 6.7% in  $\dot{V}O_2$  during steady state cycle exercise at 60-70%  $\dot{V}O_{2max}$  in glycogen depleted subjects compared with controls. In the latter study,  $\dot{V}CO_2$  was also significantly higher and R, although not reported, was calculated from their gas exchange data to be 0.92 in control compared with 0.91 in the glycogen depleted state.

Not all studies agree that oxygen consumption is higher during exercise in glycogen depleted subjects. Some have reported no significant difference in  $\dot{V}O_2$  between control and glycogen depleted subjects during incremental (McLellan and Gass, 1989b; Podolin, *et al.*, 1991) or prolonged exercise (MacLean, *et al.*, 1991). An explanation for the difference in gas exchange data reported in these studies is not readily apparent, given the fundamental difference in high energy phosphate production for different metabolic fuels (Appendix III). It can only be suggested that in some experimental conditions, as seen clearly in the MacLean, *et al.*, (1991) study, intramuscular glycogen was not as depleted as the authors intended. In the latter study, although subjects completed a strenuous exercise protocol to deplete muscle glycogen, similar to the one used in the present thesis, the exercise was followed by 2.5 days on an assigned mixed (M) or high carbohydrate (HC) diet. At the time of the experiment, muscle glycogen was not significantly different between the M or HC condition, which suggests that the mixed diet was adequate to restore muscle glycogen. In addition, no significant difference in blood glucose, lactate or the respiratory exchange ratio (R) were observed between the experimental groups, either at rest or during exercise. Obviously, care must be taken in the interpretation of data from some glycogen depletion studies.

## PLASMA VOLUME

Acute exercise results in a decrease in blood volume, or hemoconcentration. The magnitude of the plasma volume shift observed during exercise in this study agrees with the findings of Harrison (1986) and Edwards, *et al.*, (1983) for cycle ergometer exercise. In the first few minutes of exercise, hemoconcentration is attributed mainly to a rise in the hydrostatic pressure within the capillaries of active muscle, and to an increase in muscle tissue osmolality (Smith, *et al.*, 1976). Dehydration, which reduces total body water and absolute blood volume, contributes to the decrease in plasma volume in prolonged exercise. Prevention of dehydration by fluid replacement attenuates exercise hemoconcentration (Gaebelein and Senay, 1980).

## HUMORAL MEDIATORS OF VENTILATION

Glycogen depletion produced a significant alteration in certain metabolites considered pivotal to ventilatory drive during exercise. Each is discussed individually, and assessed for a contribution to the ventilatory response to exercise. The potential mediators of ventilation are then compared to the pattern of ventilation observed in response to exercise.

## AMMONIA

The present study is the first to relate exercise-induced hyperammonemia to exercise hyperpnea. Blood ammonia concentration  $[\text{NH}_3]$  increased in a slow but steady manner throughout steady state exercise in the GN condition in the present study (Fig. 20). A progressive upward drift in  $[\text{NH}_3]$  which was observed, particularly after 70 minutes of prolonged steady state exercise, was remarkably similar to the upward drift in  $\dot{V}_E$  observed at approximately the same time (Fig. 12). During prolonged exercise in glycogen depleted subjects, the group mean blood ammonia concentration was significantly greater at corresponding time points of exercise than in the control condition, although the



duration of exercise was significantly shorter in the glycogen depleted state. This result agrees with a previous finding by Broberg and Sahlin (1988) who reported a significantly greater ammonia accumulation during submaximal exercise to exhaustion in subjects who were glycogen depleted. Broberg and Sahlin observed a peak  $\text{NH}_3$  concentration of  $238 \pm 34 \mu\text{M}$  and  $263 \pm 36 \mu\text{M}$  as a result of prolonged exercise at 60-70%  $\dot{V}\text{O}_{2\text{max}}$  in control and glycogen depleted subjects respectively, compared with the peak  $\text{NH}_3$  concentration of  $144 \pm 27 \mu\text{M}$  and  $148 \pm 14 \mu\text{M}$  in similar exercise conditions in the current report. The reason for the quantitative difference in ammonia concentration between these studies may have been a result of different analysis techniques used (a flow injection technique based on a colourimetric measurement in one, compared with a spectrophotometric method using an enzyme-driven reaction to alter NADPH concentration), or the site of blood sampling (femoral venous compared with antecubital venous).

In the present study, no significant difference in blood ammonia concentration between the control and glycogen depleted subjects was observed during ramp incremental exercise to exhaustion, a result which was not anticipated based on a previous report of ammonia accumulation during prolonged exercise in glycogen depleted subjects (Broberg and Sahlin, 1988). The above finding may be rationalized, in part, by a decreased acidosis in the glycogen depleted state in response to intense exercise (Fig. 9) which would decrease the activation of AMP deaminase, and consequently lower the production of IMP and  $\text{NH}_3$  (Dudley and Terjung, 1985; Tullson and Terjung, 1991).

The reported elevation in blood  $\text{NH}_3$  concentration in response to exercise in humans varies widely in the literature. In response to prolonged exercise in healthy subjects, peak  $\text{NH}_3$  has been reported as low as  $80 \mu\text{M}$  after two hours of cycle exercise at 70%  $\dot{V}\text{O}_{2\text{max}}$  (Wagenmakers, *et al.*, 1990); approximately  $100 \mu\text{M}$  in both control and glycogen depleted subjects during submaximal exercise at 75%  $\dot{V}\text{O}_{2\text{max}}$  to exhaustion (MacLean, *et al.*, 1991);  $160 \mu\text{M}$  after forty minutes cycle exercise at 70%  $\dot{V}\text{O}_{2\text{max}}$  (Graham, *et al.*, 1987); or as high as the level cited by Broberg and Sahlin (1988) of  $238$

and 263  $\mu\text{M}$   $\text{NH}_3$  after submaximal exercise to exhaustion at 60-70%  $\dot{V}\text{O}_{2\text{max}}$ . An equally wide range of  $\text{NH}_3$  has been reported in response to incremental exercise to exhaustion, or short sprint exercise. Buono, *et al.*, (1983) observed a curvilinear increase in  $\text{NH}_3$  to a peak concentration of 100  $\mu\text{M}$  at exhaustion; after four minutes of supramaximal cycle exercise at 115%  $\dot{V}\text{O}_{2\text{max}}$ , Harris and Dudley (1989) reported a peak  $\text{NH}_3$  of 121  $\mu\text{M}$  in plasma, 136  $\mu\text{M}$  in whole blood, and 152  $\mu\text{M}$  in erythrocytes; incremental exercise to exhaustion resulted in a peak ammonia concentration of about 130  $\mu\text{M}$  in a study by Babij *et al.*, (1983), although the nature of the protocol required a total exercise duration of greater than thirty minutes; Katz, *et al.*, (1986) observed a peak arterial whole blood  $\text{NH}_3$  of 210  $\mu\text{M}$  and an arterial plasma  $\text{NH}_3$  concentration of 112  $\mu\text{M}$  following 4 min exercise at 100%  $\dot{V}\text{O}_{2\text{max}}$ ; the highest venous  $\text{NH}_3$  reported in response to incremental exercise to exhaustion was 271  $\mu\text{M}$ , reported by Banister, *et al.*, (1983). Other data have been reported as the change in ammonia concentration from rest to exercise ( $\Delta\text{NH}_3$ ) (Lo and Dudley, 1987) which cannot be directly compared with the absolute value reported in most other studies. Similarly, blood  $\text{NH}_3$  concentration at rest has been reported to range from as low as 30  $\mu\text{M}$  (MacLean, *et al.*, (1991): antecubital venous plasma) to 95  $\mu\text{M}$  (Katz, *et al.*, (1986): femoral arterial whole blood). In summary, although it is difficult to compare the absolute concentration of blood ammonia between different studies, the relative increase in blood ammonia concentration during exercise within each study is significant.

The question of whether the increase in blood ammonia concentration produced by exercise is sufficient to stimulate ventilation needs to be considered. From evidence in animal studies, in which ventilation was stimulated in response to a physiological increase in ammonia concentration of similar magnitude to that observed in exercise (Wichser and Kazemi, 1974; Dutton and Berkman, 1978), it seems reasonable to suggest that the increase in blood ammonia concentration is sufficient to contribute to the stimulation of ventilation. However, Wichser and Kazemi (1974) reported that the increase in ventilation was more closely related to an increase  $[\text{NH}_3]$  in the CSF than in arterial blood. From

these data, it was interpreted that ammonia may act centrally to stimulate ventilation. Therefore, a close relationship of a change in ventilation and blood  $[\text{NH}_3]$  would not necessarily be indicative of ammonia's concentration or action at a central ventilatory site. The absolute increase in blood ammonia concentration produced by exercise is, however, sufficiently large to suggest that it may contribute to exercise hyperpnea, both during ramp, incremental and prolonged exercise.

## POTASSIUM

In the present study venous potassium concentration  $[\text{K}^+]$  was measured (Fig. 9, Fig. 20). In response to ramp incremental exercise, venous  $[\text{K}^+]$  increased gradually with an increase in work rate, whereas during prolonged exercise, a rapid increase in  $[\text{K}^+]$  was observed at the onset of the step increase in work rate. As steady state exercise duration increased, only a marginal, non-significant upward drift in  $[\text{K}^+]$  was observed.

Exercise in the glycogen depleted state did not significantly increase the venous  $[\text{K}^+]$  compared with the control condition. This result agrees with Busse, *et al.*, (1989) who observed that the exercise-induced increase in  $[\text{K}^+]$  was similar in normal and glycogen depleted subjects, in spite of a significant difference in pH and  $[\text{La}]$  during prolonged exercise at the ventilatory threshold of human subjects. In contrast, McLellan and Gass (1989b) reported that the increase in  $[\text{K}^+]$  was significantly less in response to incremental exercise to exhaustion when a subject was glycogen depleted and eating a low carbohydrate diet, although resting  $[\text{K}^+]$  was unaffected by prior exercise or dietary manipulation.

## ARTERIAL VERSUS VENOUS POTASSIUM

Several studies on the control of breathing during exercise have suggested that arterial  $[\text{K}^+]$  plays an important role in exercise hyperpnea (Kilburn, 1966; Band, *et al.*,

1982; Linton, *et al.*, 1984; Sneyd and Wolfe, 1988; Busse, *et al.*, 1989; Paterson, *et al.*, 1989a; Newstad, *et al.*, 1990; Paterson, *et al.*, 1990; Yoshida, *et al.*, 1990). Sneyd and Wolfe (1988) compared the relationship between arterial and venous  $[K^+]$  during exercise and found that although the arterial and venous  $[K^+]$  were identical at rest, the increase in the antecubital venous blood  $[K^+]$  was less than arterial potassium during leg exercise in man, although the pattern of change was similar. When combined arm and leg exercise was performed, the increase in the arterial and venous  $[K^+]$  was identical, suggesting that inactive forearm muscle is involved in the uptake of potassium released from the leg. Newstad, *et al.*, (1990) however, in a study which reported that the increase in arterial potassium concentration was similar during cycling (leg-only) and rowing (arm and leg exercise), suggested that the uptake of potassium by resting muscle does not significantly limit the venous hyperkalemia observed during exercise. More recently, Lindinger and Sjogaard (1991) demonstrated that while the actual plasma  $[K^+]$  depends on the site of blood sampling during exercise, the pattern of change of plasma  $[K^+]$  is the same in the venous blood of contracting muscle, in arterial blood, and in venous blood of non-contracting tissues.

The 1 mM rise in venous  $[K^+]$ , observed in the present study during prolonged exercise, is similar to that reported by others using a similar exercise stimulus (Busse, *et al.*, 1989). However, ramp incremental exercise to exhaustion in this study produced an elevation of venous  $[K^+]$  closer to 1.6 mM, which is low compared with the change of 3.0 mM in human arterial  $[K^+]$  reported by Yoshida, *et al.*, (1990) in response to a similar ramp incremental protocol to exhaustion, and by Patterson, *et al.*, (1989a) following maximal exercise on a cycle ergometer. However a study by Newstad, *et al.*, (1990) clearly illustrated the inter-individual difference in the potassium response to incremental exercise to exhaustion, demonstrating a range of increase in arterial  $[K^+]$  from 1.0 to 3.0 mM in human subjects performing ramp exercise to exhaustion.

It may be assumed, therefore that the pattern of increase in venous  $[K^+]$  in the current study, although quantitatively smaller than arterial potassium, is qualitatively similar.

As discussed in the introduction, elevated blood  $[K^+]$  seems to play a strong role in exercise-induced hyperpnea. However, its role in the modulation of increased ventilation in the glycogen depleted state is uncertain.

### CATECHOLAMINES

In the present study, ramp exercise resulted in a 8-fold increase in NE in the GN condition, and 8 to 10-fold increase in the  $GD_{ACUTE}$  and  $GD_{CHRONIC}$  conditions respectively. At the end point of prolonged steady state exercise, NE had increased 7-fold from rest in the GN condition, and 6-fold from rest in the  $GD_{ACUTE}$  condition in spite of a significant reduction in exercise duration in the glycogen depleted trial. Exercise in the glycogen depleted state elicited an increase in NE that was not significantly greater than observed during control exercise, although the response tended to be greater at an equivalent work rate in ramp exercise, and at equivalent time points in prolonged steady state exercise.

A previous study in which dietary manipulation was used to reduce muscle glycogen showed that exercise in glycogen-depleted subjects elicited a significantly greater increase in NE and E compared with exercise in a normal glycogen state, although inter-individual variation is greater in the carbohydrate-poor condition (Jansson, *et al.*, 1982). Galbo, *et al.*, (1979) also reported an increased E response to exercise in glycogen depleted subjects, but found no significant enhancement of their NE response to exercise, in contrast to the results of Jansson under the same conditions. One recent study reported a decrease in the catecholamine response to incremental exercise to exhaustion in glycogen depleted subjects compared with controls (Podolin, *et al.*, 1991).

The evolution of catecholamine measurement techniques from the original bioassay to the radioimmunoassay, and more recently high performance liquid chromatography (HPLC), and the difficulty associated with each method is reflected in the range of catecholamine data published in the literature. One major difficulty in the analysis of catecholamines is their extremely low concentration in plasma. The normal, resting concentration of plasma norepinephrine and epinephrine is 1.5-2.0 nM, and 0.5 nM, respectively. At these concentrations, the HPLC technique used in the present study, which relies on electrochemical detection of catecholamines, is close to the lower limit of sensitivity of the system. The signal-to-noise ratio of the output signal is sensitive to small alterations in the mobile phase pH, flow rate, temperature, and tiny air bubbles which may become trapped in the system during the injection procedure. During extraction of the plasma sample, some of the plasma catecholamine is lost. Recovery of the internal standard in the recovery process is typically 65 to 75% (Gratzfeld-Husgen and Schuster, 1990). In addition, the extraction of a biological sample does not completely eliminate substances confounding clear discrimination of catecholamines in the chromatogram, since peaks not present in the analysis of catecholamine standards are frequently present. In the present study, a large contaminating peak with the same retention time as epinephrine (E) in the standard solution was present in many of the chromatograms of the plasma samples, which obscured the relatively small epinephrine peak. Because of this, only plasma norepinephrine (NE) concentration is reported here.

The reason for the difference in the reported findings of the relative response of plasma NE and E during exercise may reflect the sensitivity of the analytical techniques used in earlier studies, particularly in the measurement of epinephrine concentration, the difference in experimental design (ramp incremental vs step incremental vs prolonged), the mode of exercise (treadmill vs cycle ergometer), exercise duration, and the state of glycogen depletion which would be reflected in blood glucose concentration. Two studies

have critiqued the assessment of catecholamine response to exercise (Banister and Griffiths, 1972; Galbo, 1983).

A greater increase in E than NE has been observed to accompany a decrease in blood glucose during exercise (Galbo, 1977a; Galbo, *et al.*, 1979). As early as 1924, hypoglycemia was shown to induce secretion of E (Houssay, *et al.*, (1924) and Cannon, *et al.*, (1924); as cited in Euler, (1974); Euler and Luft, 1952). Crone (1963) suggested that a critical plasma glucose existed between 50 and 70 mg glucose per 100 ml blood, or 2.8 - 3.9 mM, at which E secretion was elicited. Other work has supported the concept that the secretion of catecholamines is triggered by a critical blood glucose level, rather than the rate of fall in blood glucose concentration, and that the elevation of E concentration is greater than that of NE in response to hypoglycemia (Lilavivat, *et al.*, 1981). Exercise involving more active muscle mass may also increase the catecholamine response. Kjaer, *et al.*, (1991) reported a 3- to 4-fold increase in plasma catecholamines during arm and leg exercise than during leg exercise alone, although Hooker, *et al.*, (1990) showed that the difference in the response to arm only, leg only, and arm-leg exercise was related to exercise intensity, and not the exercising muscle mass.

In the present study, the exercise-induced increase in NE concentration was not significantly greater in the glycogen depleted subjects than observed in the control state. This suggests that the observed decrease in blood glucose was not sufficient to trigger additional catecholamine secretion, or that without the analysis of E, this effect could not be detected.

There is ample evidence in the literature to suggest that norepinephrine stimulates ventilation in humans. Having few data points in for norepinephrine in the present study makes it difficult to compare its increase to the pattern of ventilation observed in response to exercise. However, it is clear that at each time point analyzed, norepinephrine is always elevated when ventilation is increased. Therefore, it is likely that the elevation of catecholamines during exercise contributes to the observed tachypneic hyperpnea.

## TEMPERATURE

In the present study, the group mean body temperature increased significantly as a result of prolonged steady state and ramp incremental exercise, in both the control and glycogen depleted subjects (Fig. 7, Fig. 18). At rest, however, there was no significant difference in body temperature between the exercise conditions.

Increased body temperature observed during exercise may contribute to the accompanying increase in ventilation. In most animals, an increase in body temperature is accompanied by an increase in ventilation (panting) as a mechanism of evaporative heat loss, comparable to sweating in man (Bligh, 1966). Hales, *et al.*, (1970) demonstrated in cross-perfused dog experiments that a passive increase in rectal temperature similar to that attained during severe exercise (i.e. 2-3 °C) was accompanied by an increase in minute ventilation from 6 to 52 l.min<sup>-1</sup>. A decrease in tidal volume was also observed, with a rapid increase in ventilatory frequency, which is characteristic of panting in animals.

In humans, elevated body temperature has been related to an increase in pulmonary ventilation in several studies (Cunningham and O'Riordan, 1957; MacDougall, *et al.*, 1974; Petersen and Vejby-Christensen, 1977; Martin, *et al.*, 1979; Martin, *et al.*, 1981). Martin, *et al.*, (1979) observed that during exercise at a constant  $\dot{V}_E$ , an increased  $T_C$  of 0.8 °C produced by exercise altered the breathing pattern by decreasing  $V_T$ , and increasing  $f$ . The decrease in  $V_T$  was associated with a shortened inspiratory time, although the drive ( $V_T/T_I$ ) and timing ( $T_I/T_{TOT}$ ) components of ventilation were unchanged by the elevation of body temperature. A subsequent study showed that passive heating sufficient to raise rectal temperature by 0.8 °C prior to exercise failed to increase the ventilatory response to exercise, although  $\dot{V}_E$  was increased at any given work rate after rectal temperature was elevated by exercise (Martin, *et al.*, 1981), suggesting that some residual effect of exercise was responsible for stimulating ventilation.

While an increase in body temperature may contribute to ventilatory drift observed during prolonged exercise, body temperature does not change quickly enough to be related



to the rapid change in  $\dot{V}_E$  observed at the onset or termination of exercise (Lambertsen, 1980), or at the ventilatory inflection points observed during ramp incremental exercise to exhaustion. In addition, the subject's core temperature was not significantly greater during exercise in the glycogen depleted, compared to the control state. Nevertheless, small changes in core temperature could potentiate the action of some of the other more potent, rapidly changing mediators of ventilation or act synergistically with them.

As with other mediators of ventilation, it is obvious that an increase in core temperature in this study, which was similar in direction to the pattern of ventilation during exercise, may have contributed to the augmented ventilatory response.

#### HYDROGEN ION CONCENTRATION

In the current study, ramp exercise in the control glycogen condition (GN) produced a characteristic decrease in blood pH with increasing exercise intensity. However in the glycogen depleted conditions, no decrease in pH was observed (Fig. 9). In response to prolonged exercise in the GN condition, a slight, transient decrease in pH was observed at the onset of the step increase in work rate, followed by a gradual, steady rise in pH to the endpoint of exercise. Constant work rate exercise in a glycogen depleted state resulted in a significant elevation in the group mean pH relative to control in prolonged steady state exercise (Fig. 20).

Heigenhauser, *et al.*, (1983) reported a similar difference in the group mean venous pH in humans during exercise in a glycogen depleted state using a comparable graded exercise protocol, although with a slower ramp function (33 watts every 4 minutes). At maximum power output during exercise, these authors reported that venous pH was  $7.26 \pm 0.03$ , and  $7.34 \pm 0.02$  respectively in their control and glycogen depleted subjects, respectively.

A decreased acidosis in glycogen depleted subjects during exercise might be expected because the major hydrogen ion source during exercise is from hydrolysis of glycolytically produced ATP (Zilva, 1978; Hochachka and Mommsen, 1983). Prior reduction of a subject's intramuscular glycogen stores would result in the increased oxidation of lipid as substrate in muscle, which would make only a minor contribution to hydrogen ion production. Although a significant body of literature exists which attributes the metabolic acidosis accompanying high intensity exercise specifically to an increment in blood lactate, it is not a major source of hydrogen ions during exercise. Its precursor, pyruvate, is already fully dissociated at physiological pH (Gevers, 1977; Jones, 1980; Alberti and Cuthbert, 1982).

Metabolic acidosis accompanying high intensity exercise is commonly cited as the cause of exercise hyperpnea. Wasserman, and colleagues, have been strong protagonists in defining a link between a non-linear increase in ventilation observed in incremental exercise to fatigue and the state of acidosis associated with lactate accumulation (Wasserman and McIlroy, 1964; Wasserman *et al.*, 1975a; Koyal *et al.*, 1976; Casaburi *et al.*, 1987; Beaver *et al.*, 1986a). Wasserman *et al.*, (1975a) demonstrated a loss of hyperventilatory response to intense exercise in humans with bilateral carotid body resection (CBX). However, no data were presented on the ventilatory response of asthmatics prior to denervation of carotid bodies. In general, asthmatics are exercise-limited by an inability to achieve high alveolar ventilation. The observed reduction in the ventilatory response to intense exercise in CBX patients was interpreted as the removal of carotid body stimulation by arterial  $[H^+]$ . This analysis, however, should be reconsidered since it is well documented that potassium (Band *et al.*, 1985; Linton and Band, 1985; Band and Linton, 1986; Paterson and Nye, 1988), catecholamines (Joels and White, 1968), and temperature (Eyzaguirre and Lewin, 1961; McQueen and Eyzaguirre, 1974) also affect carotid body activity.

## LACTATE

A 40 to 50% reduction in the peak lactate concentration in the blood was observed in response to exercise in a glycogen depleted state in the present study. These results agree with previous findings of (Karlsson and Saltin, 1971; Costill, *et al.*, 1971; Asmussen, *et al.*, 1974; Segal and Brooks, 1979; Jansson, 1980; Hughes, *et al.*, 1982; Hargreaves, *et al.*, 1984; Neary, *et al.*, 1985).

The present finding of a lower lactate concentration accompanying exercise in a glycogen depleted state may reflect an increased lactate utilization. However, it has been clearly demonstrated that the intramuscular concentration of lactate is reduced in glycogen depleted subjects (Jansson, 1980; Jacobs, 1981b). Factors which may contribute to this are factors which also regulate glycolysis. These include (i) altered substrate availability and circulating hormone concentration which would affect substrate selection (Galbo, *et al.*, 1977b; Coyle, *et al.*, 1983; Kirwan, *et al.*, 1990), and (ii) a continued inhibition of glycolysis by increased citrate concentration (which potentiates the allosteric inhibition of PFK by ATP) as a result of increased lipid oxidation (Newsholme and Leech, 1983). It has also been suggested that prior glycogen depletion of skeletal muscle may affect activation of phosphorylase b by a change in the local concentration  $\text{Ca}^{2+}$ , Pi, ATP or AMP within the tissue (Gollnick, *et al.*, 1978; Newsholme and Leech, 1983; Ren and Hultman, 1990).

## ROLE OF THE CAROTID BODY TO THE STIMULATION OF VENTILATION

Two assumptions on the role of the carotid body must be aired before the importance of humoral mediators, specifically  $[\text{H}^+]$ ,  $[\text{K}^+]$ ,  $\text{PaO}_2$  and  $\text{PaCO}_2$ , acting at peripheral chemoreceptors to stimulate the ventilatory response to exercise is decided. The first assumes that an increased discharge from the intact carotid body stimulates ventilation. The second suggests that the controlled stimulation of ventilation observed at rest, when a change in one mediator of ventilation at a time may be controlled, is similar to the response

observed during exercise. Wasserman *et al.* (1975a) observed that carotid body denervated asthmatics did not hyperventilate in response to intense exercise, suggesting the importance of the carotid body in this response. Band *et al.* (1985) also reported that bilateral denervation of the carotid and aortic sinus nerves abolished the effect of elevated  $[K^+]$  on the stimulation of ventilation. However, both the importance, and the role of the intact carotid body during exercise has been questioned. Pan *et al.* (1986), observed an increased ventilatory response to exercise in ponies with denervated carotid bodies, and concluded that carotid chemoreceptors were not critical for hyperventilation during exercise in ponies. Since carotid body denervation did not eliminate or even attenuate the respiratory compensation for metabolic acidosis during exercise in these animals, it was suggested that input from the carotid body might play a role in dampening the neurogenic exercise drive to hyperventilation (Pan *et al.*, 1986). If the carotid body chemoreceptors are not important in ventilatory drive during exercise, this would reduce the importance of  $[H^+]$ ,  $[K^+]$ , norepinephrine, and possibly  $\Delta T_C$ , as potential mediators of exercise hyperpnea. A opposing point of view, however, would propose that the role of carotid body input to the central respiratory integrator assumes more importance as exercise progresses. The importance of the carotid body illustrated by Wasserman *et al.* (1975a) lies in the ventilatory response to high intensity exercise, or exercise above the anaerobic threshold. Evidence by Hansen *et al.* (1982), who attenuated the ventilatory drift of prolonged exercise by the administration of hyperoxia which would silence the carotid body, also suggested that the carotid body is important in the mediation of ventilatory drift during prolonged exercise.

In the present study, this controversy cannot be resolved. However, the suggestion that carotid body input may be important in "dampening" the ventilatory response to exercise is interesting.

## A COMPARISON OF VENTILATION WITH THE MEDIATORS OF VENTILATION

In order to relate the pattern of ventilation to the pattern of change in any proposed mediator of ventilation, the physiological correctness of the relationship must be evident. While each of the known or proposed mediators of ventilation monitored in this study (ammonia, potassium, pH, norepinephrine, and core temperature) has been studied for their individual effect in the stimulation of ventilation, it is also appropriate to consider that the ventilatory response to exercise may result from a summation of input from all, or a number mediators, whose individual contribution varies depending on the presence or absence of other stimuli. A different ratio of mediators may itself influence the ventilatory response.

Both the increased hyperpnea observed during exercise in glycogen depleted subjects, and the positive ventilatory drift accompanying prolonged constant work rate exercise, was mediated by an increase in breathing frequency (Fig. 11 and 22). In response to ramp incremental exercise, an increase in ventilation was associated with an increase in  $[\text{NH}_3]$ ,  $[\text{K}^+]$ , norepinephrine, and  $\Delta T_C$ , and a decrease in pH in the control (GN) exercise condition. While the increase in  $[\text{NH}_3]$  may be viewed as an appropriate stimulus producing the observed change in ventilation, it is inappropriate to rule out the contribution of any one of the other mediators noted, which might have contributed to the overall ventilatory response. At the point of a non-linear increase in ventilation as exercise intensity increased, the pattern of change of each of the ventilatory mediators was similar to the increase in ventilation, although pH was most sensitive and responsive to the change at this time, in the GN condition.

However, the ventilatory response to exercise in the glycogen depleted state suggested that an increase in  $[\text{H}^+]$  is not obligatory to the hyperventilatory response to exercise. Glycogen depletion was used in this study to allow comparison of ammonia production and a different level of ventilation for an equivalent work rate and level of  $\text{CO}_2$  production. A significantly greater group mean ventilation observed during ramp exercise

in the glycogen depleted state was associated with a relative alkalosis (Fig. 11), suggesting that  $[H^+]$  could not have stimulated the hyperpnea at this time. However, in the absence of acidosis, none of the remaining mediators of ventilation ( $[NH_3]$ ,  $[K^+]$ , norepinephrine, and  $\Delta T_C$ ) could clearly account for the tachypneic hyperpnea observed in the glycogen depleted condition.

In response to prolonged constant work rate exercise, no single mediator of ventilation could account for the ventilatory drift in the control condition as well as the significantly higher ventilation in the glycogen depleted state. While there was a similarity in the overall pattern of ventilation to the observed change in ammonia concentration, the relationship was not always compatible. For example when ventilation tended to be greater from the onset of exercise in the glycogen depleted condition, ammonia concentration was not different from the control condition. When ventilation remained at a steady state value up to the 70th minute of exercise,  $[NH_3]$  drifted slowly but progressively upwards. However, two specific points support the role that ammonia may play in the stimulation of ventilation during prolonged exercise. The secondary rise in  $[NH_3]$  observed after 70 min was the only ventilatory mediator measured which changed sufficiently to account for the concomitant positive ventilatory drift. Also, in the glycogen depleted state, the response of  $[NH_3]$  was the only mediator appropriately and significantly different from the control condition to account for the significant increase in ventilation observed.

Of course, this does not imply that  $[K^+]$ , norepinephrine, and  $\Delta T_C$  did not contribute to the stimulation of ventilation in either ramp or constant work rate exercise. It has been well documented that an increase in  $[K^+]$ , norepinephrine, and  $\Delta T_C$  all stimulate ventilation. However, in the present study, neither  $[K^+]$  nor  $\Delta T_C$  were significantly different in response to either of the exercise protocols used in the glycogen depleted, compared with the control condition. Therefore, unless there is a specific increase in the sensitivity of the receptor for  $[K^+]$  or  $\Delta T_C$  in the glycogen depleted state, the significantly

higher ventilation observed in the glycogen depleted subject cannot result from either  $[K^+]$  or  $\Delta T_C$ .

Another possibility is that in the absence of one mediator, in this case acidosis, the relative contribution of the remaining mediators of ventilation changed. Lockwood *et al.* (1980) reported that the rate of ammonia transport across the blood brain barrier increased in mild alkalosis in experimental animals. If this result can be applied to the exercising human, then in the glycogen depleted state a greater proportion of blood ammonia would have access to the central nervous system. The present study did not allow this possibility to be tested, but this hypothesis would allow ammonia to stimulate a centrally-mediated increase in ventilation, in the absence of an excessive increase in arterial ammonia concentration.

#### **RATING OF PERCEIVED EXERTION, AND ITS CORRELATION WITH PHYSIOLOGICAL AND BIOCHEMICAL MEASURES DURING EXERCISE**

The subjective measurement of rating of perceived exertion (RPE) is an accepted and simple method of determining a subject's exercise intensity (Borg, 1982). In the present study, this measure, recorded at regular intervals throughout exercise, was significantly greater during exercise in glycogen depleted subjects at every corresponding time point in both prolonged (Fig. 18) and ramp incremental exercise (Fig. 7).

This is the first time that a rating of perceived exertion has been correlated with  $\Delta[NH_3]$  during exercise. In the context of the present thesis, it was of interest to show how the relationship of RPE with  $\Delta[NH_3]$  compared with other physiological and biochemical measures related to RPE, and whether  $\Delta[NH_3]$  was consistently related to RPE in every exercise condition. During prolonged exercise, RPE was strongly correlated with  $\Delta[NH_3]$  in both the **GN** ( $r=0.71$ ) and **GD<sub>ACUTE</sub>** ( $r=0.85$ ) condition. This correlation between  $\Delta[NH_3]$  and RPE was somewhat reduced ( $r=0.49$ ) when only the latter stage of exercise in the **GN** condition was considered (Table 19). In the ramp exercise protocol,

RPE was consistently correlated with  $\Delta[\text{NH}_3]$  between 0.57 and 0.72 in the three exercise conditions, but this was not as strong as the correlation of RPE with other physiological and biochemical markers (Table 12).

As previously mentioned, the Borg scale of perceived exertion has been shown to correlate between 0.80 and 0.90 with heart rate, oxygen uptake, and lactate accumulation (Borg, 1982), and is extensively used in exercise testing (ACSM, 1991), exercise prescription (Pollock, *et al.*, 1986), and in the quantification of energy expenditure (Cardio Stress Inc, 1988).

The relationship between RPE and pulmonary ventilation is also of interest. Martin, *et al.* (1981) suggested that if the sensed level of breathing is an important part of the overall perception of exertion during prolonged exercise, or if significant ventilatory muscle fatigue occurs during heavy exercise, then a rising  $\Delta\dot{V}_E$  could reduce exercise tolerance. In the present study, RPE was more closely correlated with  $\Delta\dot{V}_E$  during ramp incremental exercise (0.84 - 0.93), than during prolonged steady state exercise (0.62 - 0.69). Both the present, and previous evidence suggests that ventilatory function and/or discomfort contributes to perceived exertion, although the exact aspect of ventilation that is sensed is unclear (Noble *et al.*, 1973; Robertson, 1982; Demello *et al.*, 1987).

Perception of effort increases with prolonged exercise duration (Fig. 18) or increasing exercise intensity (Fig. 7). Since the perception of effort during exercise, although subjective, reflects a central cognitive process encompassing many contributing factors, it may also be a measure of central fatigue or a loss of willingness to continue with exercise. Based on experimental evidence of brain ammonia metabolism (c.f. Cooper and Plum, 1987, for a recent review), Banister and Cameron (1990) have postulated that an exercise-induced elevation in ammonia production contributes to central fatigue, possibly by a reduction of high energy phosphate concentration, or through an alteration in neurotransmitter concentration in the brain, with the development of symptoms of ammonia toxicity. The relationship between blood ammonia concentration, its movement



across the blood brain barrier, and its interaction with endogenous brain biochemistry and physiology was discussed in the introduction. This is a speculative, but interesting hypothesis which may be pursued in the future when technological progress will allow the determination of brain metabolites during exercise.

## LIMITATIONS OF THE PRESENT STUDY

There are several limitations to the conclusions drawn from these experiments. Subjects in this study were males, aged 22 to 30, and represented a relatively fit, recreational athletic population. The results of the study, therefore, pertain to similar populations. Extrapolation of the results of this study to other healthy, or patient population, may not be appropriate.

A major limitation of this study is the small sample size. Extreme data from one subject might bias the group mean data. In addition, inter-individual variability may have obscured a significant relationship which would have been more obvious with a larger number of subjects. Caution must be exercised, therefore, in the interpretation of these data.

It was not possible to establish unequivocally a dose-response relationship between ammonia and exercise hyperpnea from this study. This results from inter-individual differences in ammonia accumulation in the blood, and possibly differences in the sensitivity of the ventilatory response of each subject to ammonia. The net accumulation of ammonia in the blood reflects both continuous production of ammonia and its release from skeletal muscle, together with its continuous uptake and buffering in various body tissues. Thus from the results of this study, it is not possible to predict a specific level of increased pulmonary ventilation from a specific hyperammonemia. Also, the source of ammonia production during exercise may have varied under different exercise conditions, from predominantly purine nucleotide cycle production (ramp exercise), to a significant contribution from amino acid catabolism (prolonged exercise).

It is well documented that many other variables (for example pH,  $K^+$ , core temperature, and catecholamines) contribute to ventilatory stimulation during exercise. In the present study, it was not possible to selectively eliminate each of these confounding variables, or to control their elevation one at a time. Thus, while the pattern of ventilation

may be related to the change in ammonia concentration during exercise, the continued presence of other confounding ventilatory stimulants precludes the identification of ammonia as the primary mediator of ventilation.

While the pattern of ventilation observed is comparable to the pattern of change in ammonia concentration, and that of several other potential mediators of ventilation, it was not possible to suggest a cause-and-effect relationship based on this observation alone. Because all variables were time-dependent, the underlying cause of their elevation may have been the exercise stimulus itself, or other factors not measured in this study. It may be suggested, for example, that the increased work rate or onset of skeletal muscle fatigue results in the selective recruitment of an increased number of motor units. By co-activation, or cortical irradiation to central respiratory centres, an increase in motor unit recruitment may have stimulated ventilation, independent of humoral input. Nevertheless, one may only suggest a temporal relationship between the change in ammonia concentration and the stimulation of ventilation from the present experiments.

In the present study, the contribution of ammonia to the control of ventilation in humans was considered during exercise. However, the control of ventilation during exercise may not be the same as in the resting subject. It was previously suggested that the sensitivity of peripheral chemoreceptors is increased in exercise (Cunningham *et al.*, 1986; Paterson *et al.*, 1989a). If the gain in peripheral chemoreceptor control is variable during exercise, then the response to the same stimulus will change depending of the intensity or duration of exercise, and would be very difficult to measure. This would complicate any interpretation of a dose-response relationship between ventilation and its potential mediators.

The present study used glycogen depletion to enhance ammonia production at an equivalent work rate, while concomitantly reducing the hydrogen ion concentration ( $H^+$ ) as a possible mediator of ventilation. The study did not consider the possibility that

by attenuating acidosis, the relative contribution of other mediators of ventilation may be altered.

It is also possible that the glycogen depletion model may produce a different metabolic and hormonal response to exercise, as a result of a change in the fuel substrate, as indicated by the reduction in the mean R in the glycogen depleted state. The higher pH evident during exercise in glycogen depleted subjects, particularly at the time of tachypneic drift and hyperpnea, also suggests that the local environment surrounding peripheral chemoreceptors was altered. This may have affected the interaction of ventilatory mediators at ventilatory receptors. Thus, the relative sensitivity of a receptor site may be affected by a local change in the metabolic environment, and the relative importance of each mediator of ventilation may change if its access to a receptor is altered by its movement between tissue compartments.

Although it is well documented that ammonia stimulates ventilation in an anaesthetized animal model, and that ammonia crosses the blood brain barrier, the actual receptor site for ventilatory stimulation by ammonia and its mechanism of action are unknown. Evidence in the literature suggests that ammonia acts centrally in the stimulation of ventilation. It has also been suggested that the central action of ammonia on ventilation may be through its conversion to glutamate or glutamine. Venous ammonia concentration, as measured in the present study, is far removed from a proposed central receptor site for ammonia. Therefore, the pattern of venous ammonia concentration may not reflect the change in ammonia concentration at or near its receptor site. Since it remains uncertain whether ammonia is directly neuroactive, or whether it contributes to the stimulation of ventilation indirectly by altering the concentration of related excitatory (glutamate) or inhibitory (GABA) neurotransmitters, further experiments need to be done which can follow the movement of ammonia between tissue compartments before its role in the stimulation of ventilation during exercise is understood.

In order to compare the pattern of change in ventilation more effectively with potential mediators of ventilation during exercise, more frequent blood samples are required. It is recommended that future studies should sample blood every 15 to 30 seconds, particularly at times of rapid increases in ventilation, or at ventilatory threshold. Lack of data density at such points in the present study limits the comparison between any inflection point in ventilation and the accompanying change in humoral mediators of ventilation.

Venous blood samples were taken in the present study for the analysis of blood metabolites. This is an important limitation in this study, since the known peripheral chemoreceptors are located in the arterial system, and there is an arterio-venous difference in the concentration of several mediators of ventilation. Thus, while the pattern of change of arterial and venous mediators of ventilation may be similar, the absolute value representing the actual stimulus at a receptor site, is quantitatively different.

Rectal temperature was measured to indicate core temperature during exercise. This site may have a slower response time, and may not be reflective of a more rapid change in temperature in the blood, which would be detected at peripheral chemoreceptors sensitive to temperature change.

The selection of exercise work rate for each subject depended on their individual maximum exercise capacity. For the ramp protocol, the incremental work rate was set at 20 or 30 watts per minute, representing an individual increment of 6.6 to 7.5 %  $WR_{MAX}$  per minute. This was preferable to selecting a single ramp increment of 30 watts per minute, which would have represented an increment of 7.5 to 10 %  $WR_{MAX}$  per minute. However, although the rate of increment represented a similar proportion of  $WR_{MAX}$  for each individual, it has been well documented that ramp slope affects the transient kinetic response of  $\dot{V}_E$ ,  $\dot{V}O_2$ ,  $\dot{V}CO_2$ . Therefore, it may not be appropriate to compare these data with other incremental work rate tests. In addition, the rate of the ramp incremental test was selected based on the individual's maximum exercise capacity in a normal, control

glycogen condition. Given that each subject fatigued at a lower work rate in the glycogen depleted state, the rate of ramp slope must be relatively higher in the glycogen depleted subject. However, the choice made in the present study was to make a comparison between absolute work rates, although it is possible that the rate of change of each potential mediator of ventilation was affected by the relative rate of change in the exercise stimulus.

Similarly, prolonged exercise was completed at 50%  $WR_{MAX}$  based on results of ramp exercise in a control glycogen condition. In the glycogen depleted state, the work rate probably reflected a relatively larger proportion of the individual's exercise capacity, and the ventilatory response observed during control work rate exercise in the glycogen depleted subject may have reflect this. The purpose of selecting 50%  $WR_{MAX}$  was to achieve a steady state condition. Because of the obvious fatigue, higher heart rate response to exercise, higher rating of perceived exertion, and probable greater recruitment of motor units to achieve the same work rate, a steady state work rate may not have been achieved in the glycogen depleted state. Again, a temporal comparison of data in the normal and glycogen depleted condition may not be appropriate, since the relative exercise stimulus is probable different.

An endpoint value was determined in each study to allow comparison of each variable at the endpoint in the control and glycogen depleted state, which could not otherwise be compared since the group mean exercise time was longer in the control glycogen protocol, than in the glycogen depleted state. In the ramp protocol, endpoint represented exhaustion in all conditions, although time to exhaustion was approximately one minute shorter during exercise in the glycogen depleted state compared with the control condition. However, in the constant work rate protocol, endpoint represented exhaustion in all 5 subjects in the glycogen depleted condition, whereas 4 of 5 subjects were able to complete 90 minutes of the constant work rate protocol in the control glycogen condition. Therefore, it is arguable that the endpoint is the appropriate point of comparison to make in the prolonged exercise test.

While it was speculated from the results of this study that a change in ventilatory sensitivity may have resulted from stimulation of receptors through the direct neuroactive properties of ammonia, or through its metabolic conversion to other neurotransmitters, it was not possible to follow the movement of ammonia between tissue compartments, or to monitor its conversion to other metabolites. From these data, therefore, it is not possible to suggest a mechanism of action of endogenously produced ammonia in the stimulation of ventilation. The results of this study cannot discount the involvement of increased core temperature, elevated catecholamine concentration, or a rise in potassium ion concentration in the stimulation of ventilation during exercise.

The investigation has, however, been valuable in providing support for the hypothesis that ammonia may contribute to the stimulation of ventilation during exercise, and deserves further study.

## CONCLUSIONS

In this thesis, the relationship between ammonia, known to stimulate ventilation at rest in humans and animals, was compared with the ventilatory response to exercise. Other "traditional" mediators of ventilation, including  $[H^+]$ ,  $[K^+]$ , norepinephrine, and core temperature, were also examined in relation to the pattern of ventilation observed. To investigate this relationship, two series of physical exercise were completed, incorporating a glycogen depletion model into the exercise protocol to allow a comparison of different levels of ventilation, and presumably a different ventilatory drive, at an equivalent work rate.

The results of this dissertation have demonstrated that venous ammonia concentration, investigated for the first time as a potential mediator of exercise hyperpnea, is related temporally to the pattern of ventilation during exercise, although other mediators of ventilation were not eliminated as possible contributors to ventilation by the experimental design.

From the results of this study, the following observations were made.

1. The ventilatory response ( $\dot{V}_E$ ) to exercise in glycogen depleted subjects was increased, relative to exercise in a control glycogen condition. The strategy to achieve a greater  $\dot{V}_E$  depended on the mode of exercise. During ramp exercise, the relative hyperventilation resulted primarily from an increase in  $f$  with no change in  $V_T$ . During prolonged exercise in the glycogen depleted subject, the relative hyperventilation resulted from an even greater increase in  $f$ , required to offset a progressive fall in  $V_T$ .
2. The glycogen depletion model is useful to study ventilation in an intact subject because it allows a different ventilation at the same work rate to be compared. There is obvious redundancy in the control of ventilation during exercise, since exercise hyperpnea is present even in the absence of known ventilatory stimulants. For example, during ramp exercise in the glycogen depleted subject, and throughout prolonged exercise, pH



was not a major facilitator of ventilatory drive. Thus,  $[H^+]$  was not obligatory to the hyperpnea observed in the glycogen depleted subject, but it does not discount the possibility that it may be an important input to ventilatory drive in the intact individual.

3. It may be argued that ammonia's position as a ventilatory stimulant is strengthened in the absence of metabolic acidosis, particularly in prolonged exercise. In this instance, when pH is not a primary candidate as a ventilatory stimulant, the response of  $K^+$  and  $T_c$  to exercise were unchanged in the glycogen depleted subject relative to the control condition, whereas  $NH_3$  was significantly elevated, and temporally related, to the observed increase in  $\dot{V}_E$ .
4. There appeared to be an altered sensitivity in the ventilatory system in a glycogen depleted subject, suggested by:
  - (i) the observed shift in the Euler plots ( $V_T$ - $T_I$ - $T_E$  diagrams) in both ramp incremental and prolonged exercise.
  - (ii) the observed increase in  $\dot{V}_E/\dot{V}CO_2$  at the same work rate during exercise in the glycogen depleted state.

The results of this study support the following conclusions.

1. Ammonia is a potential contributor to the stimulation of  $\dot{V}_E$  during exercise.
2. In the glycogen depleted subject, the contribution of acidosis as a primary stimulant of ventilation is reduced, yet hyperpnea is present during prolonged or intense exercise. This supports the theory of redundancy in respiratory control. Of the mediators of ventilation measured in the present study,  $NH_3$ ,  $K^+$ , norepinephrine,  $\Delta T_c$ , and pH all may contribute to ventilatory drive during exercise.

3. The close temporal relationship between  $\text{NH}_3$  and  $\dot{V}_E$  during exercise in both normal and glycogen depleted subjects supports the hypothesis that  $\text{NH}_3$  may stimulate ventilation, especially during heavy exercise. Patterson and Nye (1988) suggested that  $\text{K}^+$  may increase the sensitivity of the carotid body during short term exercise. It is conceivable that  $\text{NH}_3$ , which is both (i) neuroactive and (ii) contributing to central neurotransmitter metabolism, may also contribute to an increase in sensitivity of the ventilatory response to exercise. Other mediators of ventilation, however, must also be considered.

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## Appendix I

### Abbreviations

ACSM	American College of Sports Medicine
AgCl	Silver Chloride
AMP	Adenosine Monophosphate
AT	Anaerobic Threshold
ATP	Adenosine Triphosphate
BBB	Blood Brain Barrier
BCAA	Branched Chain Amino Acid
BSA	Body Surface Area
BTPS	Body Temperature and Pressure, Saturated with Water Vapour
BV	Blood Volume
CBX	Carotid Body Denervated
CC	Cerebral Cortex
CIA	Central Inspiratory Activity
CNS	Central Nervous System
CO <sub>2</sub>	Carbon Dioxide
CSF	Cerebrospinal Fluid
CV	Cell Volume
DHBA	Dihydroxybenzamine
DRG	Dorsal Respiratory Group
E	Epinephrine
ECF	Extracellular Fluid
EDTA	Ethylenediamine tetra-acetic acid
<i>f</i>	Breathing Frequency
FEV <sub>1</sub>	Forced Expiratory Volume in one second
FVC	Forced Vital Capacity
GAD	Glutamate Decarboxylase
GABA	Gamma Amino Butyric Acid
GD <sub>ACUTE</sub>	Acute Glycogen Depleted Condition
GD <sub>CHRONIC</sub>	Chronic Glycogen Depleted Condition
GN	Normal Glycogen Condition
H <sup>+</sup>	Hydrogen Ion
HClO <sub>4</sub> <sup>-</sup>	Perchloric Acid

$\text{HCO}_3^-$	Bicarbonate Ion
HPLC	High Performance Liquid Chromatography
IMP	Inosine Monophosphate
IPSPs	Inhibitory Post Synaptic Potential
$\text{K}^+$	Potassium Ion
KCl	Potassium Chloride
LT	Lactate Threshold
MVV	Maximum Voluntary Ventilation
NADH	Nicotine Adenine Dinucleotide (reduced)
NAD	Nicotine Adenine Dinucleotide (oxidized)
NaOH	Sodium Hydroxide
NE	Norepinephrine
NE:E	Norepinephrine:Epinephrine Ratio
$\text{NH}_3$	Ammonia
$\text{NH}_4^+$	Ammonium Ion
$\text{O}_2$	Oxygen
$\text{PaCO}_2$	Partial Pressure of Carbon Dioxide (arterial)
$\text{PaO}_2$	Partial Pressure of Oxygen (arterial)
$P_{\text{ETCO}_2}$	End-tidal Pressure of Carbon Dioxide
$P_{\text{ETO}_2}$	End-tidal Pressure of Oxygen
pH	Hydrogen Ion Concentration, expressed as the negative logarithm, using the Henderson-Hasselbalch Equation
PV	Plasma Volume
R	Respiratory Exchange Ratio ( $\dot{V}\text{CO}_2/\dot{V}\text{O}_2$ )
RH	Relative Humidity
RPE	Rating of Perceived Exertion
RPM	Revolutions Per Minute
STPD	Standard Temperature and Pressure, Dry (0°C, 760 mm Hg, dry)
$T_c$	Core Temperature
$T_E$	Expiratory Time
$T_I$	Inspiratory Time
$T_{\text{TOT}}$	Time of one breathing cycle (1/f)
$T_I/T_{\text{TOT}}$	Inspiratory Timing
$\dot{V}\text{CO}_2$	Volume of Carbon Dioxide Per Minute
$\dot{V}_E$	Ventilatory Volume Per Minute
$\dot{V}_E/\dot{V}\text{CO}_2$	Ventilatory Equivalent for Carbon Dioxide Production



$\dot{V}_E/\dot{V}O_2$	Ventilatory Equivalent for Oxygen Consumption
$\dot{V}O_2$	Volume of Oxygen Per Minute
VRG	Ventral Respiratory Group
VT	Ventilatory Threshold
$V_T$	Tidal Volume
$V_T/T_I$	Drive Component of Ventilation
$WR_{MAX}$	Maximum Work Rate

## Appendix II

**Table 7 Coefficient of Variation in each Biochemical Analysis**

The following table is a summary of the method used in each biochemical analysis, and the coefficient of variation of the method determined in this study.

Analyses	Technique	Reference	Coefficient of Variation * %
Lactate	Spectrophotometric, lactate dehydrogenase	Sigma, 1989	3.1
Ammonia	Spectrophotometric, glutamate dehydrogenase	DCL, 1989	4.6
Glucose	Colourimetric, glucose oxidase	Marks and Dawson, 1965	5.4
	Hexokinase and glucose-6-phosphate dehydrogenase	Schmidt, 1961	Normal - 1.9 High - 1.5
Catecholamines (Norepinephrine, Epinephrine)	High performance liquid chromatography	Mefford, <i>et al.</i> , 1981	NE: 7.4 E#: 11.3
Hematocrit	Centrifugation	Coulter, 1988	0.9
Hemoglobin	Cyanmethaemoglobin	Lynch, 1976	0.7
Potassium	Ion-selective electrode		Normal - 1.5 High - 1.1
pH	Blood Gas Analyzer, CIBA Coming 178, pH electrode	Forster, <i>et al.</i> , 1972	0.4

\*Coefficient of variation (CV) was based on duplicate analyses from the same blood sample.  
# Determined on duplicate analyses of standard solutions

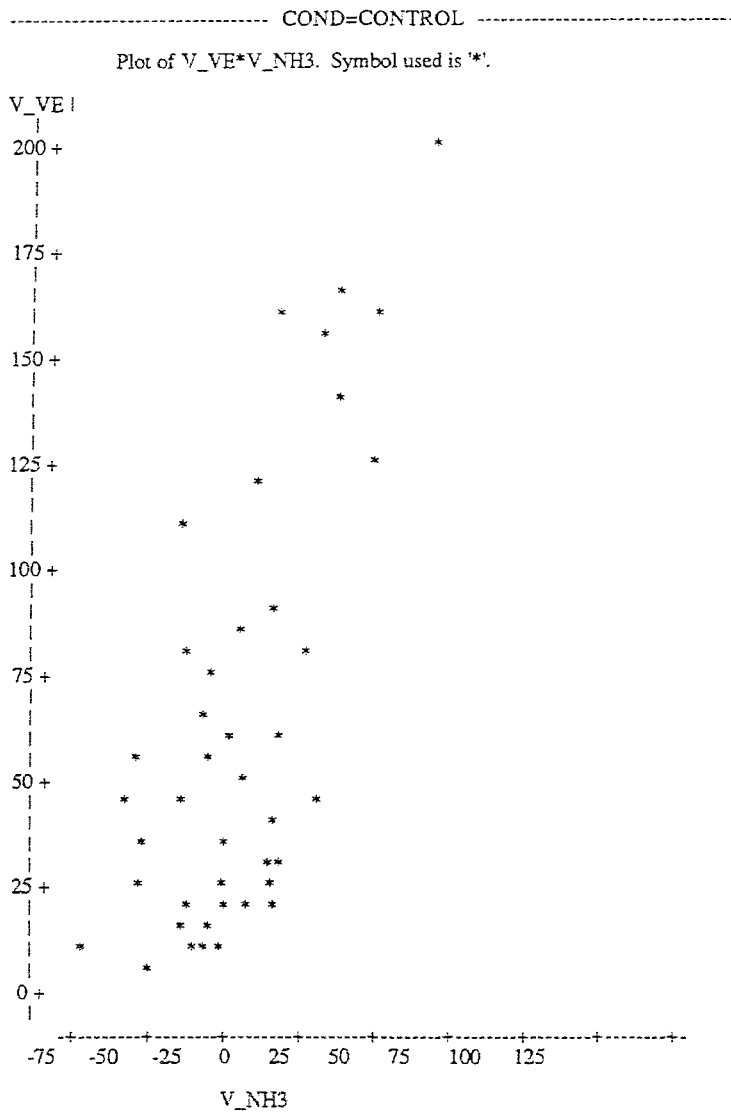
## Appendix III

### EFFECT OF SUBSTRATE SUPPLY ON ENERGY YIELD

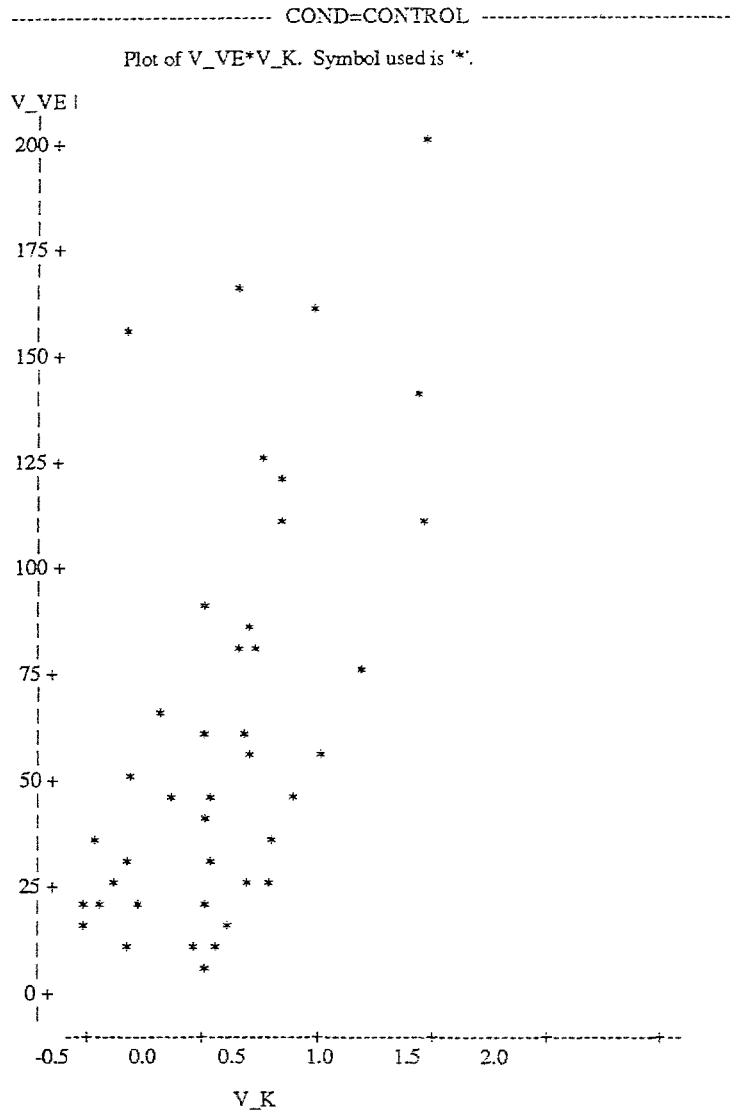
In the current study, a significant increase in  $\dot{V}O_2$  at an equivalent work rate in both ramp and prolonged exercise was observed when subjects were glycogen depleted compared with their control condition. There was no similar significant increase in  $\dot{V}CO_2$  between the glycogen depletion state and the control condition. The R value, an index of muscle substrate metabolism, was significantly lower in each glycogen depleted state indicating a shift towards increased fat oxidation by the working muscle (Jansson, 1980). Because of the shift in the energy substrate from carbohydrate to a larger proportion of fat, more  $O_2$  would have had to be consumed to generate an equivalent amount of ATP to match the energy demand of exercise. The ATP yield per mole of  $O_2$  is less for fats than for carbohydrates. If stored muscle glycogen is the carbohydrate source, a high-energy phosphate yield of 6.2 moles per mole of  $O_2$  consumed is expected. Stored fat, on the other hand, yields 5.6 moles of high-energy phosphate per mole of  $O_2$  consumed (McGilvery, 1970; Newsholme and Leech, 1983). Thus for an equivalent energy demand,  $O_2$  consumption would be expected to increase by 6.2/5.6, or 19.6%, if pure fat instead of pure carbohydrate was used as the only fuel substrate. An accurate estimate of the proportion of fat and carbohydrate utilized at any point of the study was not possible because the necessary condition of a metabolic steady state, with no lactate production, was not met.

## Appendix IV

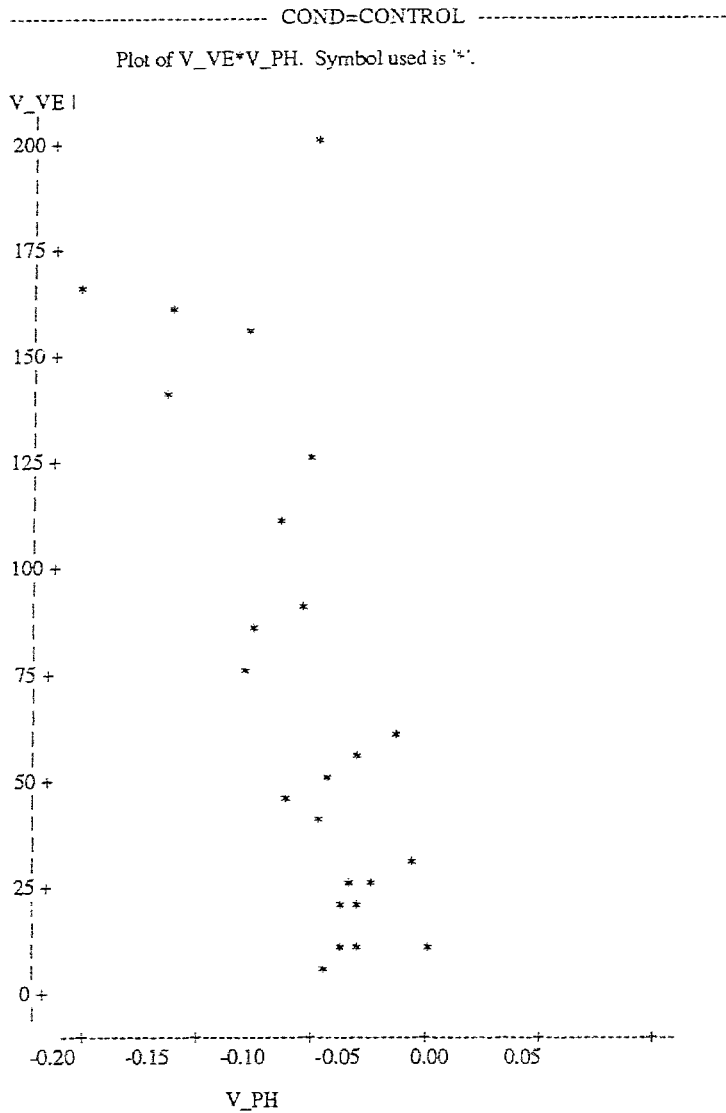
Individual scattergrams showing the relationship between the change in ventilation and the change in potential mediators of ventilation (ammonia, potassium, pH, core temperature, and norepinephrine) measured in this study.



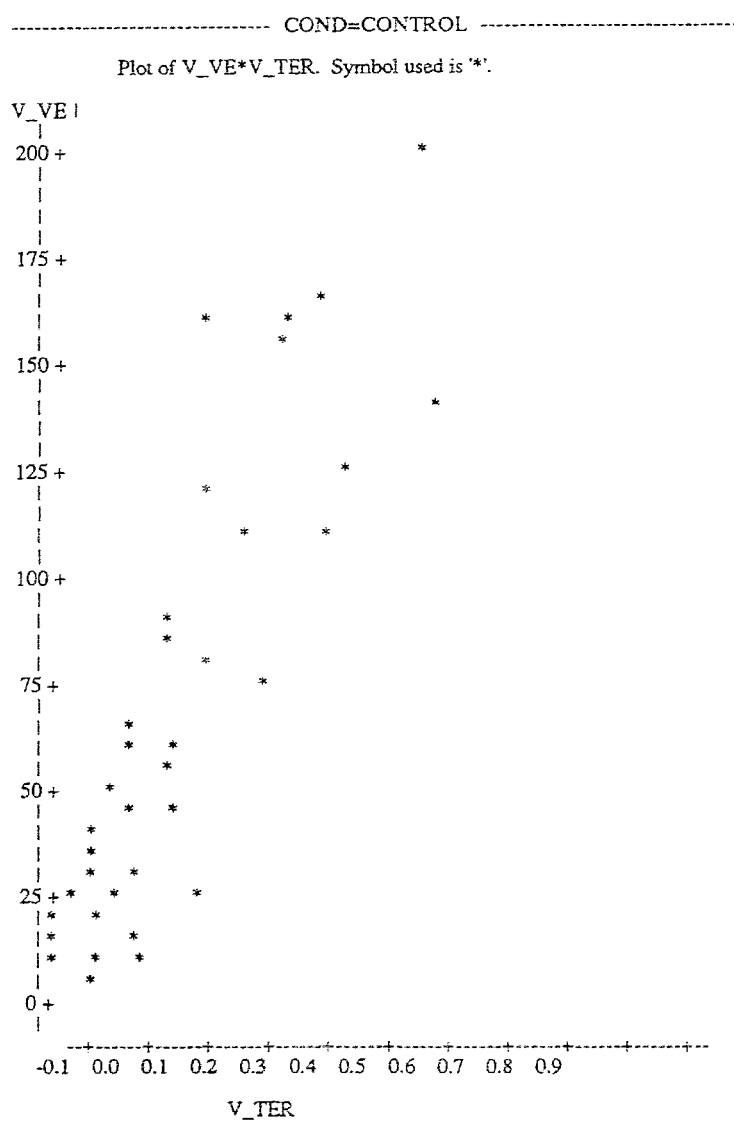
**Figure 24** Scattergram showing the individual relationship between the change in ventilation ( $l \cdot \text{min}^{-1}$ ) and the change in ammonia concentration ( $l \cdot \text{min}^{-1}$ ) in response to ramp exercise in the GN condition.



**Figure 25** Scattergram showing the individual relationship between the change in ventilation ( $\text{l}\cdot\text{min}^{-1}$ ) and the change in potassium concentration (mM) in response to ramp exercise in the GN condition.

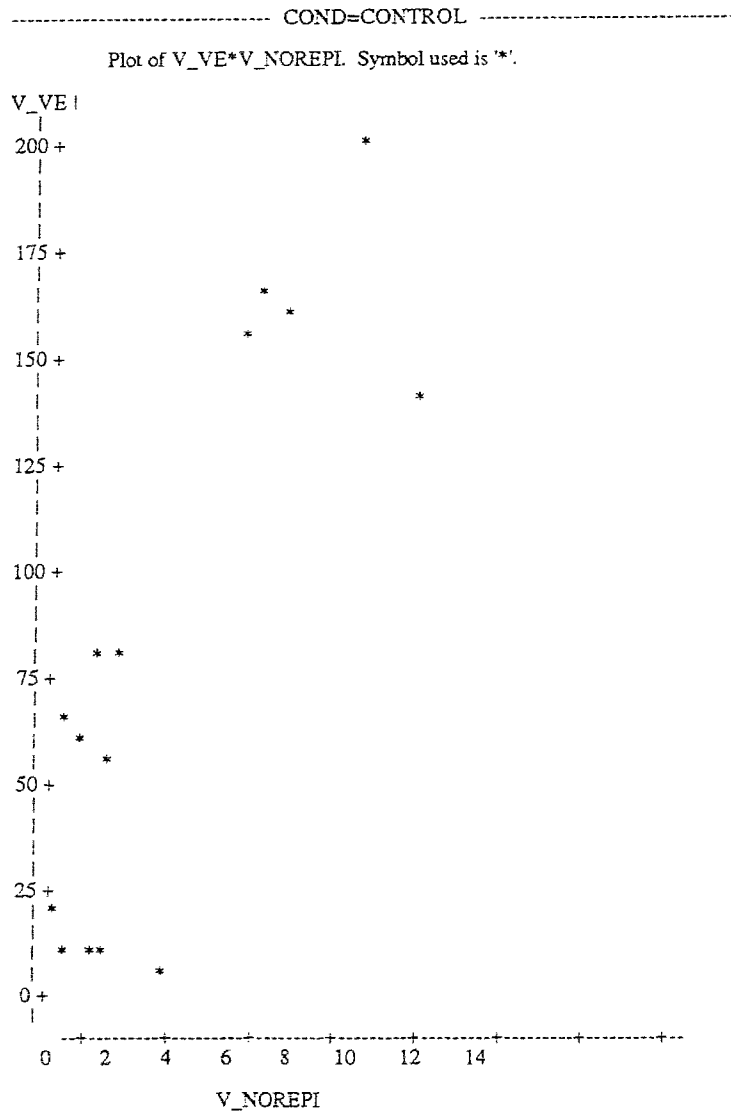


**Figure 26** Scattergram showing the individual relationship between the change in ventilation ( $l \cdot \text{min}^{-1}$ ) and the change in pH in response to ramp exercise in the GN condition.

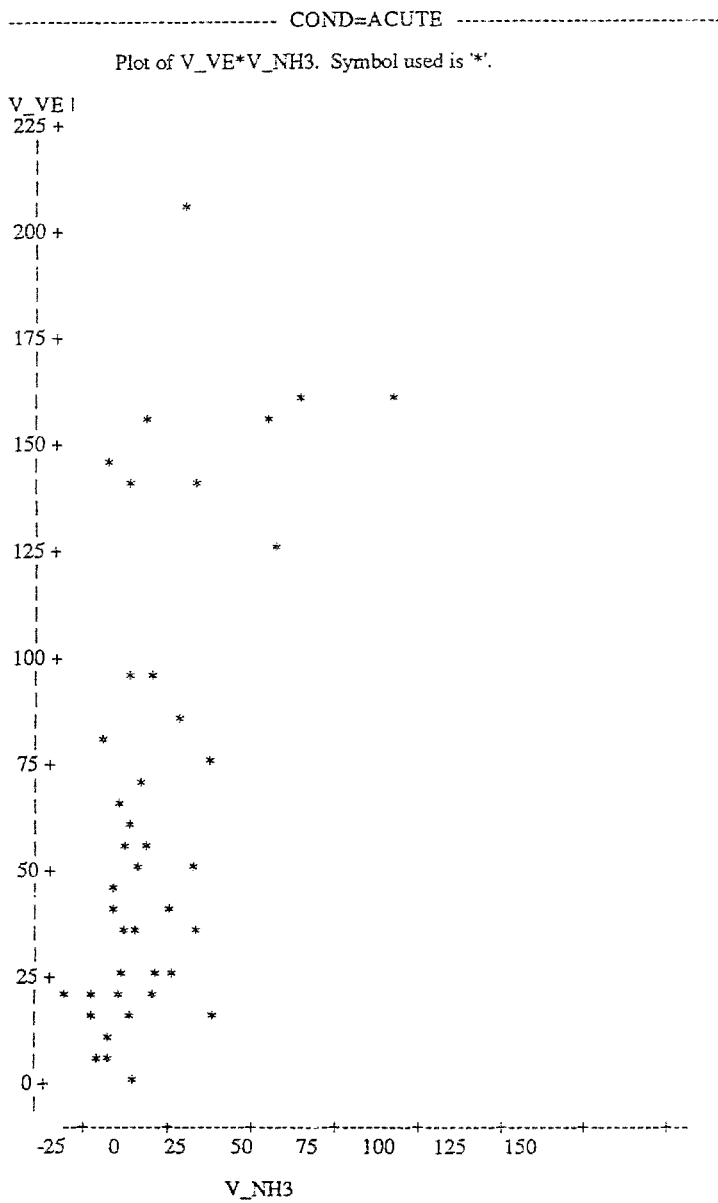


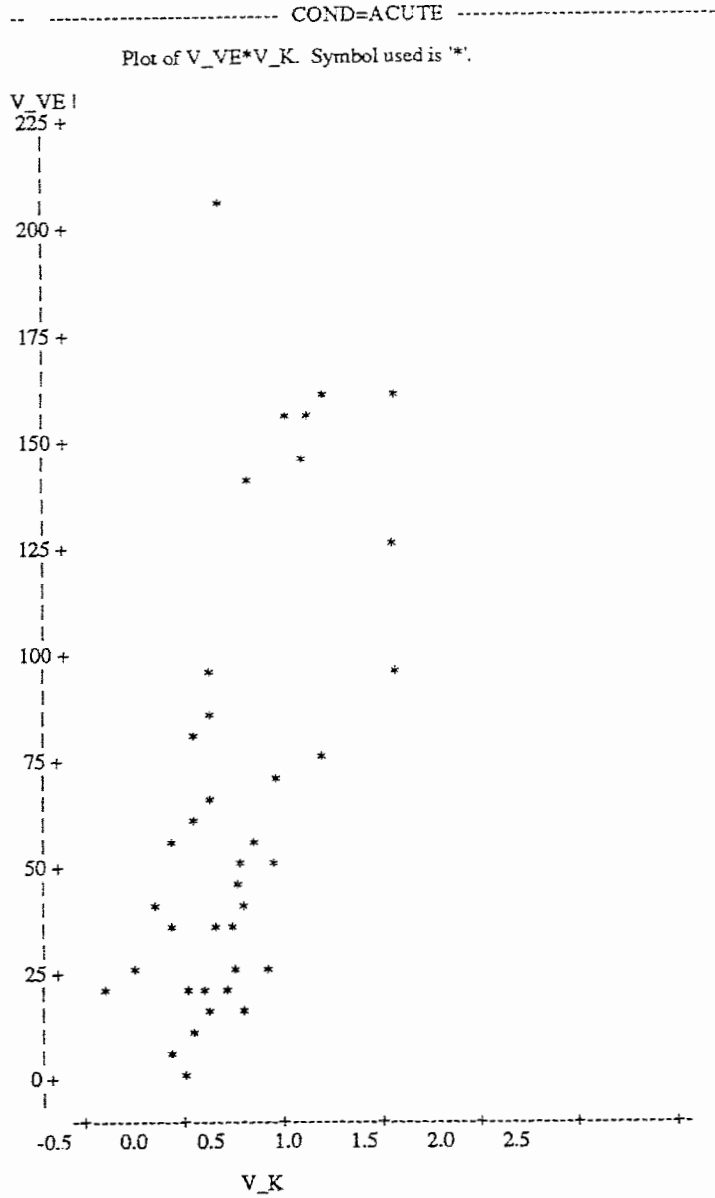
**Figure 27** Scattergram showing the individual relationship between the change in ventilation (l·min<sup>-1</sup>) and the change in core temperature (°C) in response to ramp exercise in the GN condition.



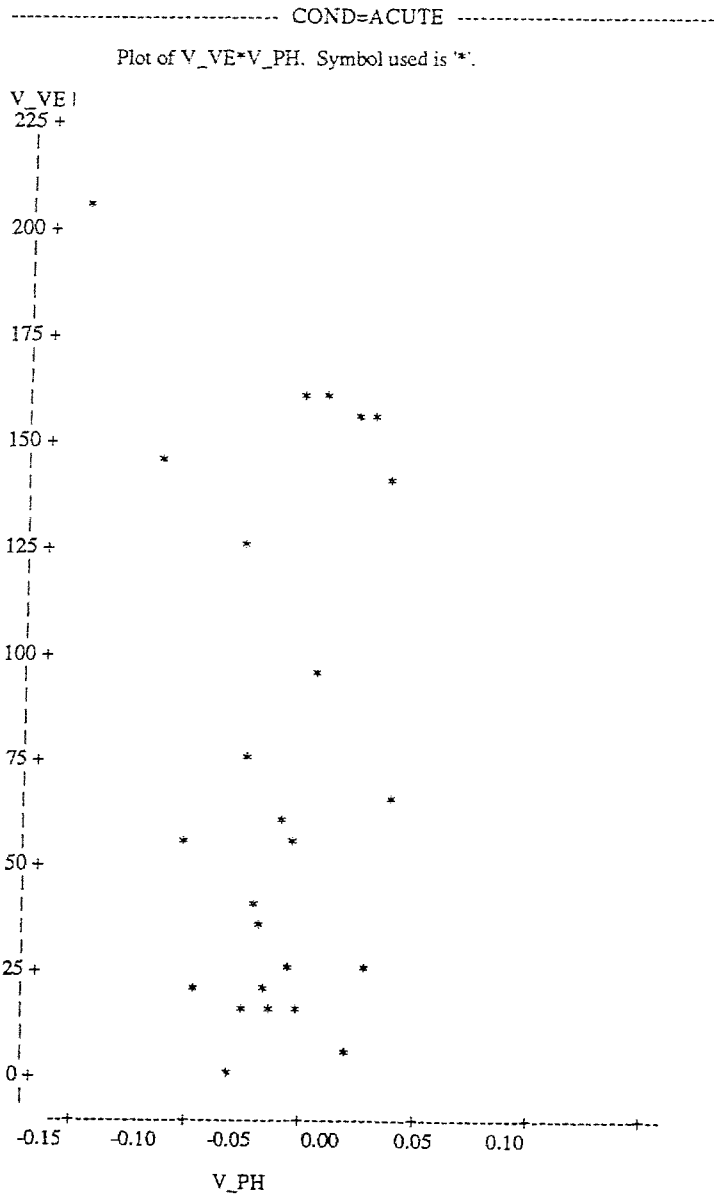


**Figure 28** Scattergram showing the individual relationship between the change in ventilation ( $\text{l}\cdot\text{min}^{-1}$ ) and the change in norepinephrine concentration (nM) in response to ramp exercise in the GN condition.

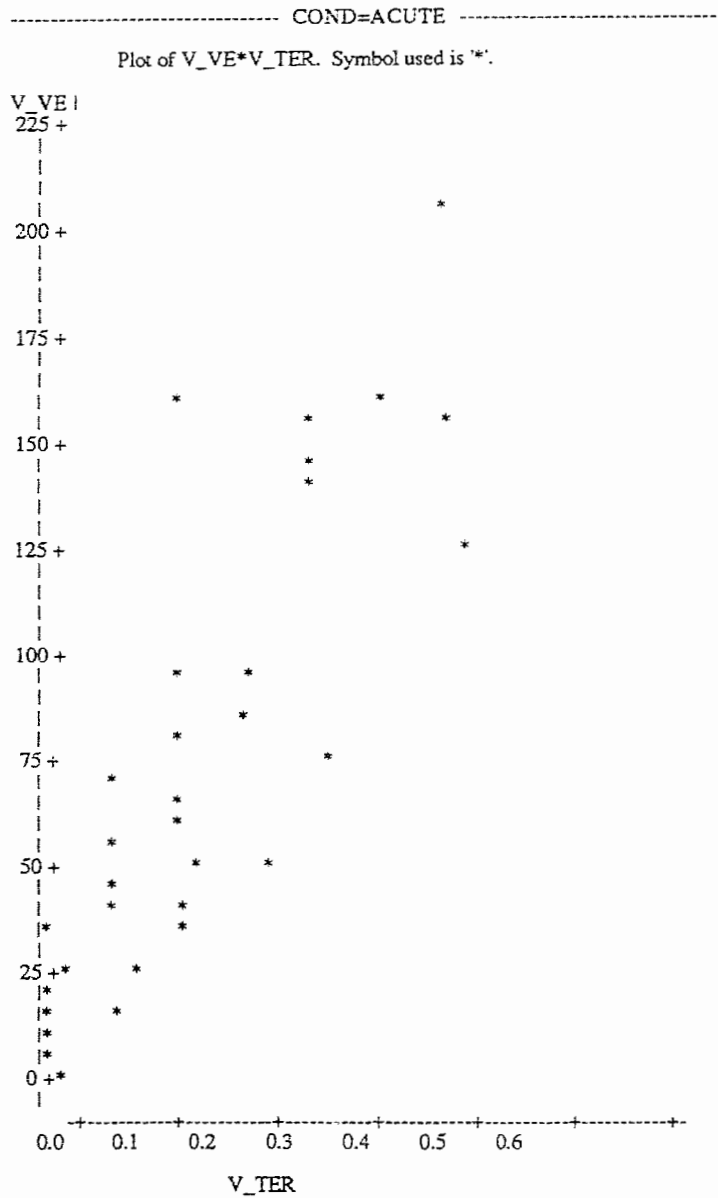




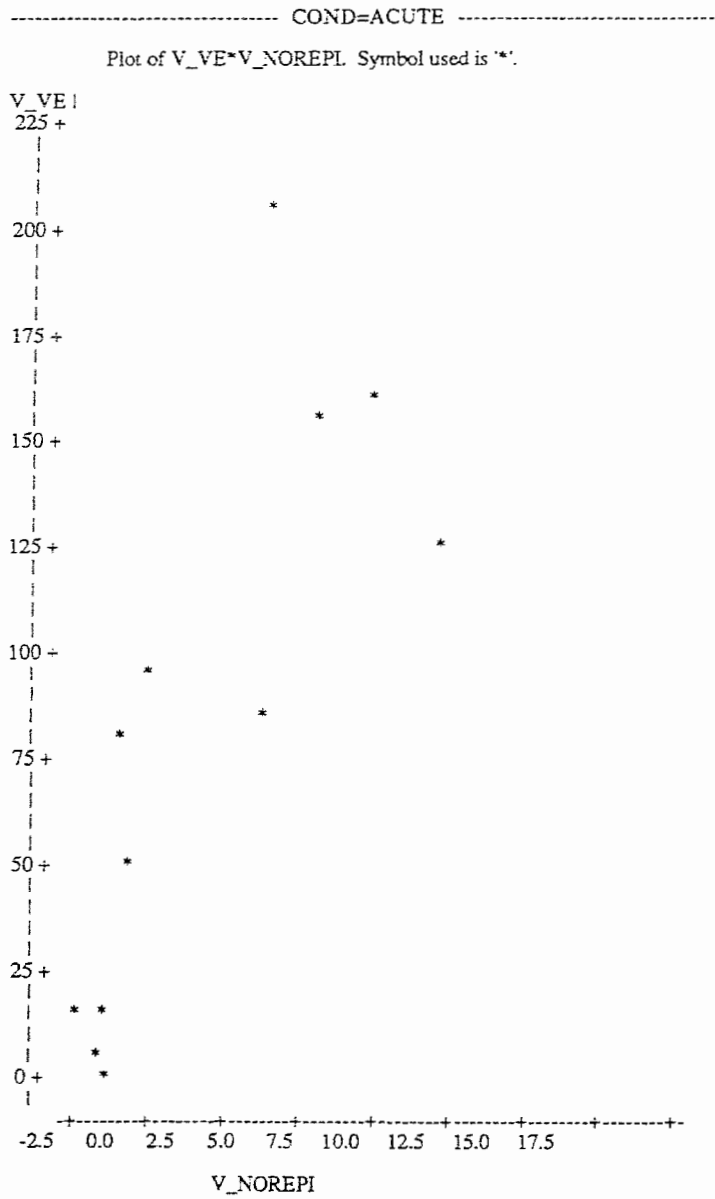
**Figure 30** Scattergram showing the individual relationship between the change in ventilation ( $\text{l}\cdot\text{min}^{-1}$ ) and the change in potassium concentration (mM) in response to ramp exercise in the  $\text{GD}_{\text{ACUTE}}$  condition.



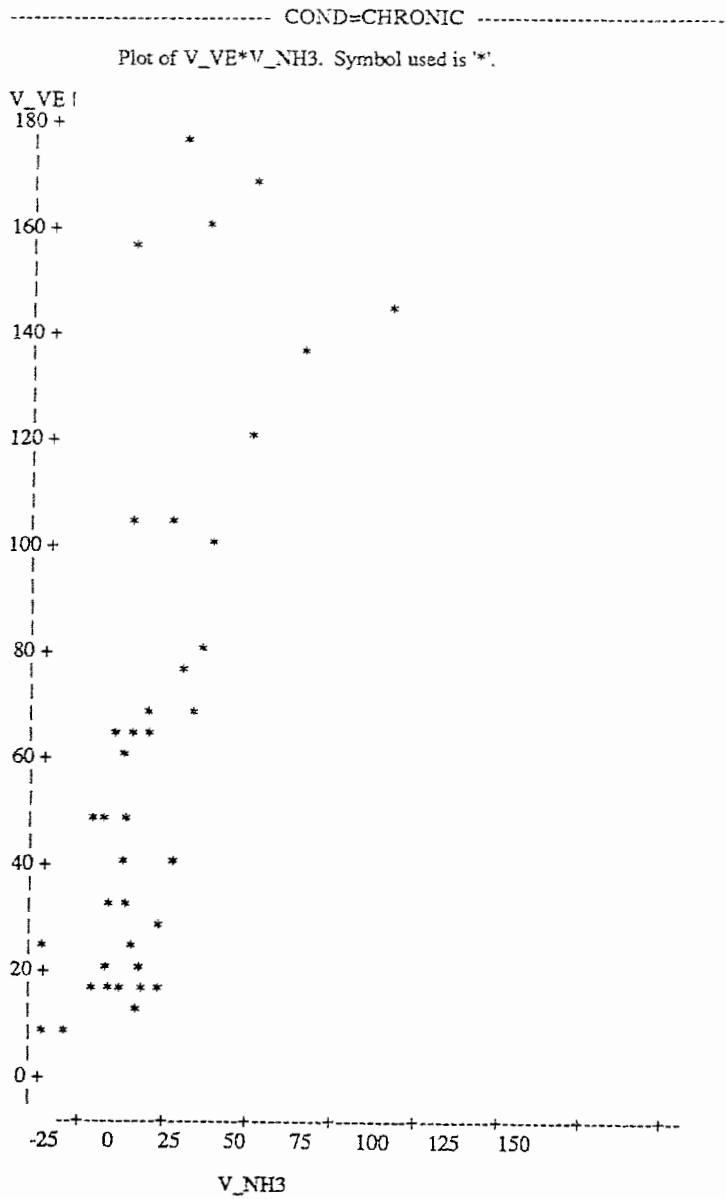
**Figure 31** Scattergram showing the individual relationship between the change in ventilation ( $l \cdot \text{min}^{-1}$ ) and the change in pH in response to ramp exercise in the  $GD_{ACUTE}$  condition.



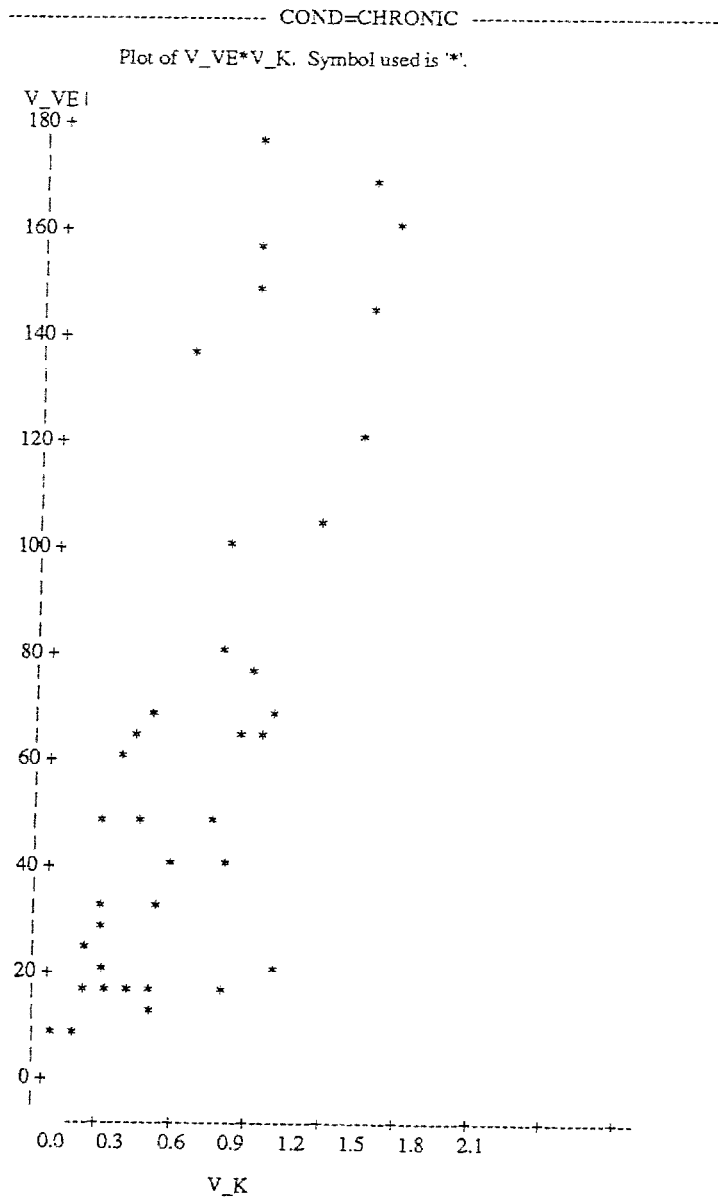
**Figure 32** Scattergram showing the individual relationship between the change in ventilation ( $l \cdot \text{min}^{-1}$ ) and the change in core temperature ( $^{\circ}\text{C}$ ) in response to ramp exercise in the  $\text{GD}_{\text{ACUTE}}$  condition.



**Figure 33** Scattergram showing the individual relationship between the change in ventilation ( $\text{l}\cdot\text{min}^{-1}$ ) and the change in norepinephrine concentration (nM) in response to ramp exercise in the  $\text{GD}_{\text{ACUTE}}$  condition.

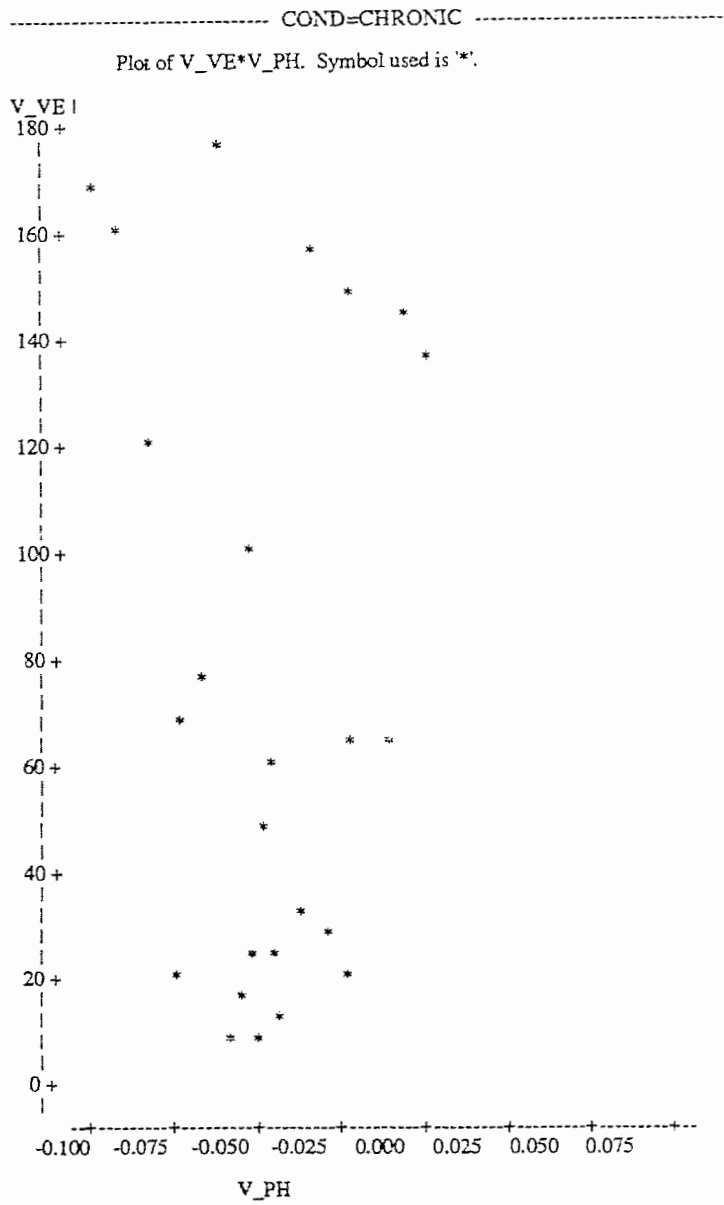


**Figure 34** Scattergram showing the individual relationship between the change in ventilation (l·min<sup>-1</sup>) and the change in ammonia concentration (l·min<sup>-1</sup>) in response to ramp exercise in the GD<sub>CHRONIC</sub> condition.

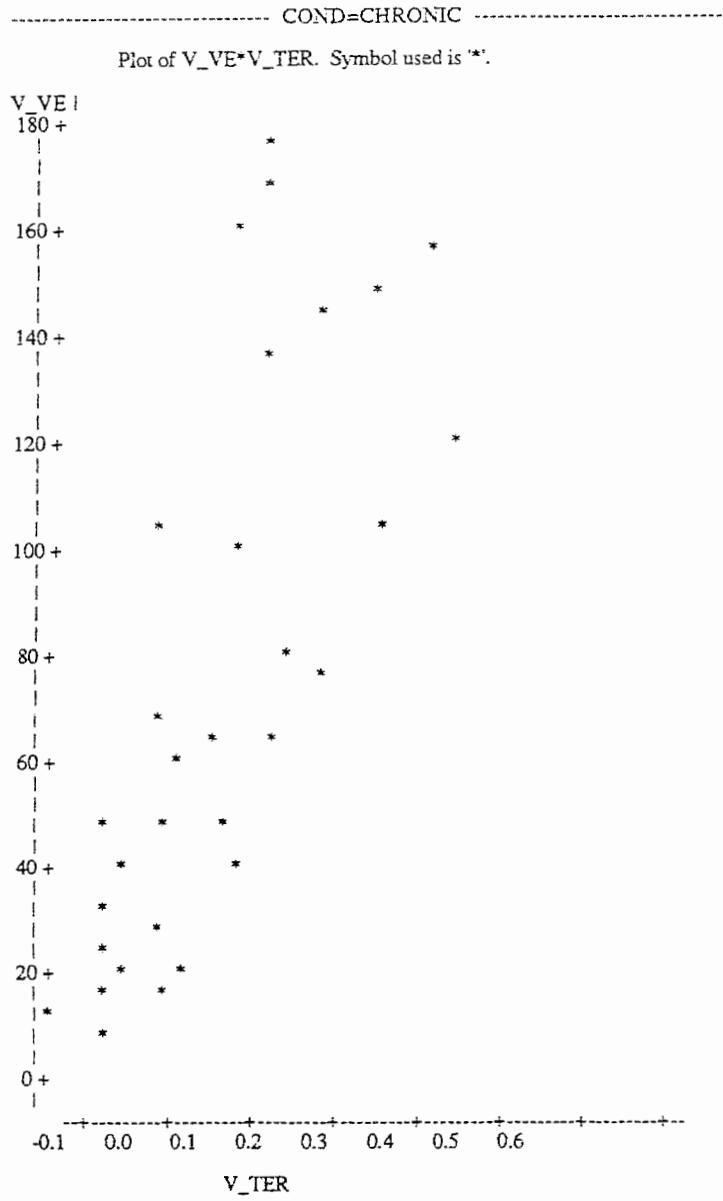


**Figure 35** Scattergram showing the individual relationship between the change in ventilation ( $l \cdot \text{min}^{-1}$ ) and the change in potassium concentration (mM) in response to ramp exercise in the  $GD_{\text{CHRONIC}}$  condition.

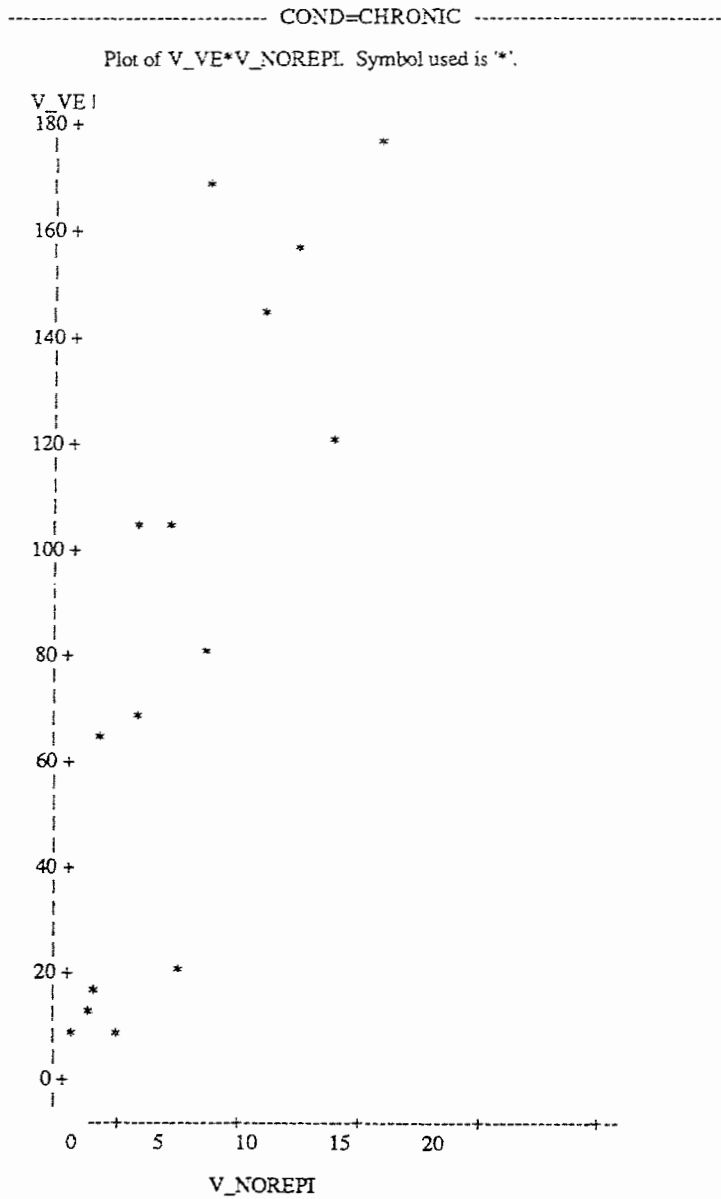




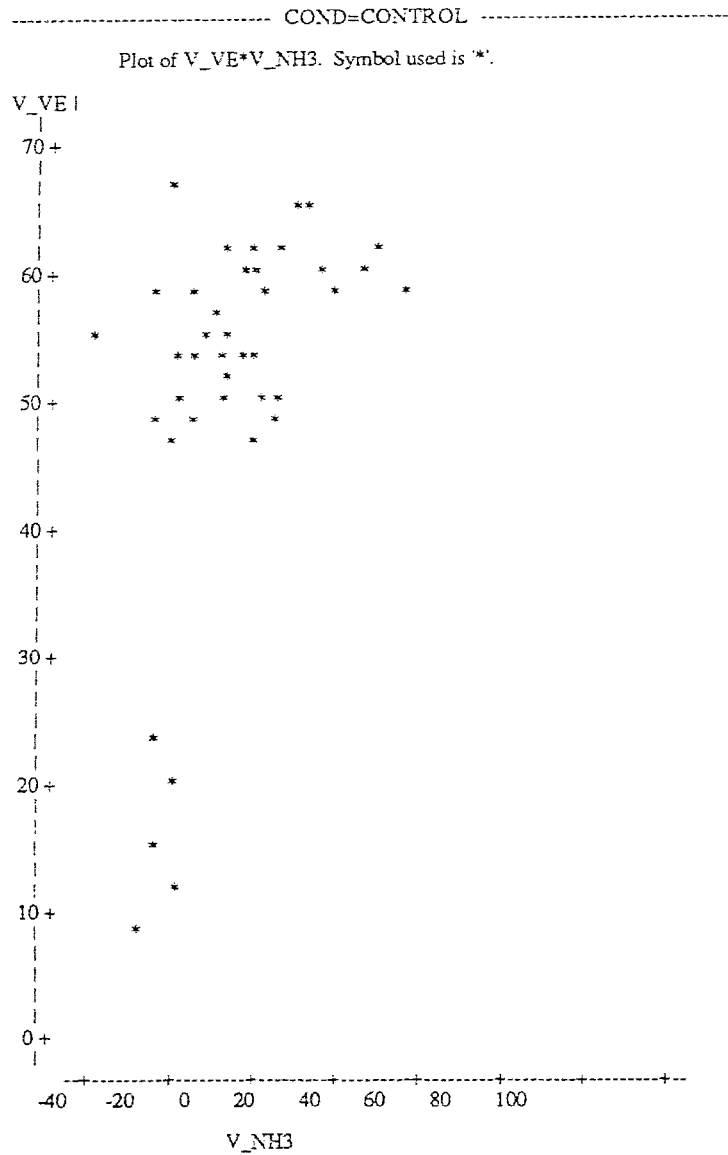
**Figure 36** Scattergram showing the individual relationship between the change in ventilation ( $l \cdot \text{min}^{-1}$ ) and the change in pH in response to ramp exercise in the GD<sub>CHRONIC</sub> condition.



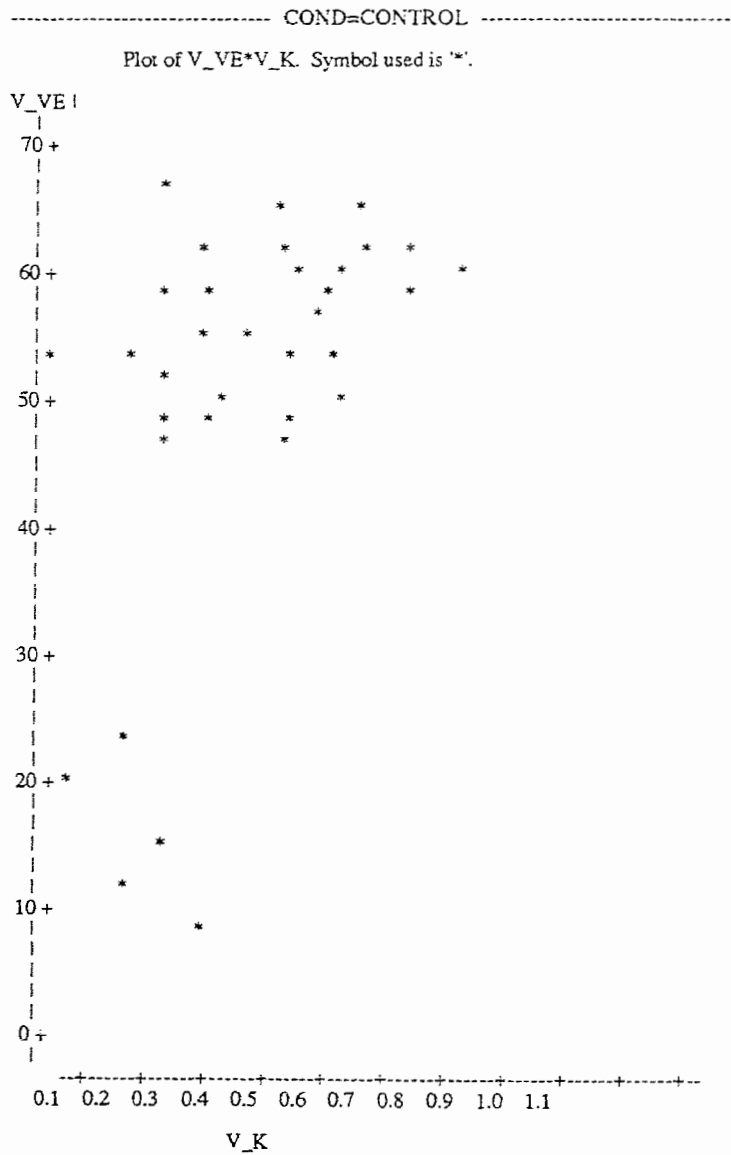
**Figure 37** Scattergram showing the individual relationship between the change in ventilation ( $\text{l}\cdot\text{min}^{-1}$ ) and the change in core temperature ( $^{\circ}\text{C}$ ) in response to ramp exercise in the  $\text{GD}_{\text{CHRONIC}}$  condition.



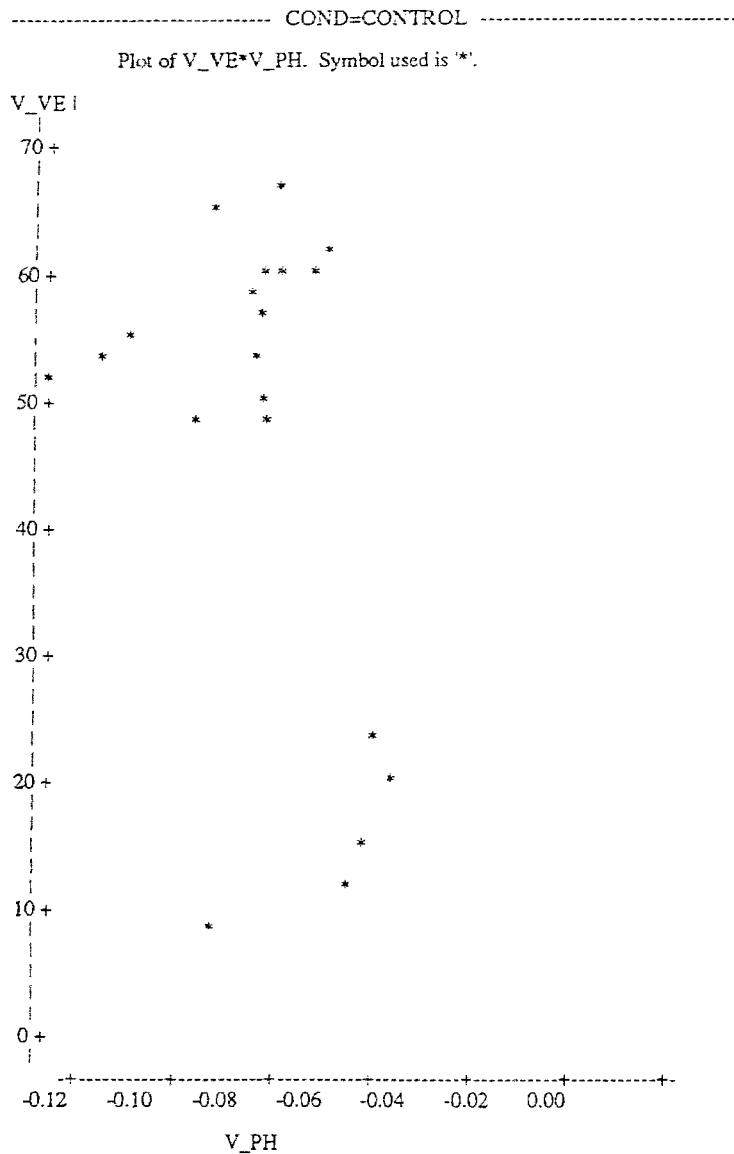
**Figure 38** Scattergram showing the individual relationship between the change in ventilation ( $l \cdot \text{min}^{-1}$ ) and the change in norepinephrine (nM) concentration in response to ramp exercise in the  $GD_{\text{CHRONIC}}$  condition.



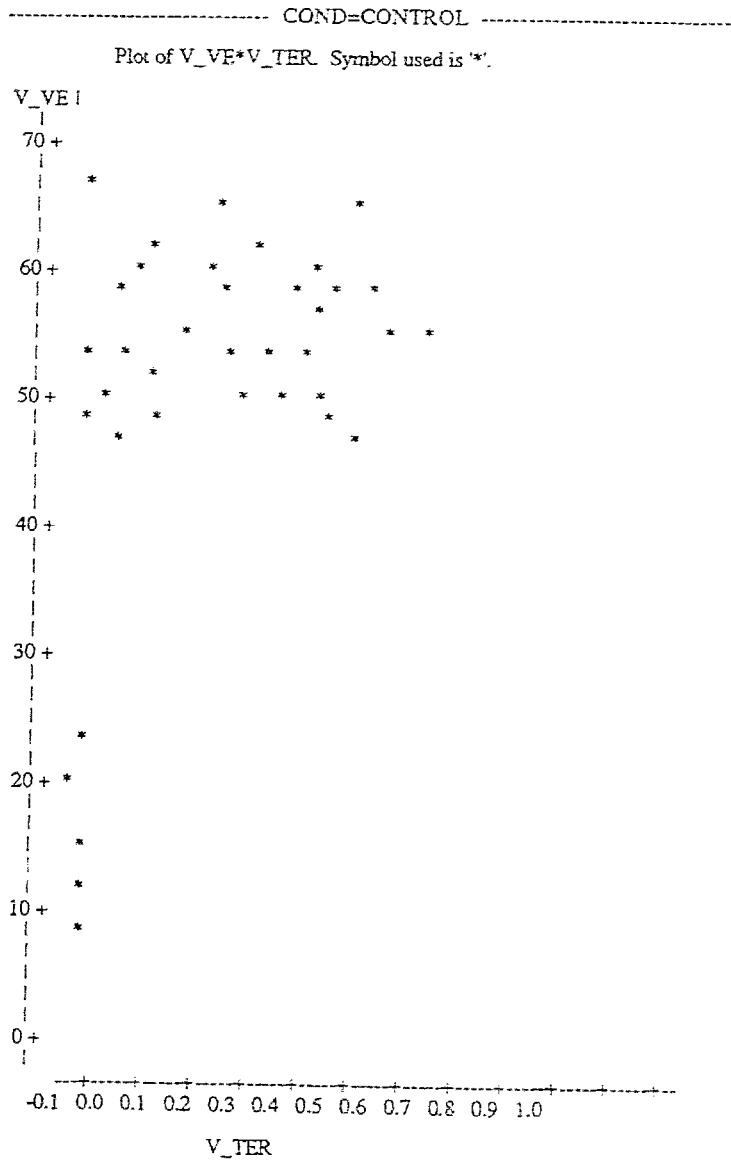
**Figure 39** Scattergram showing the individual relationship between the change in ventilation ( $l \cdot \text{min}^{-1}$ ) and the change in ammonia concentration ( $l \cdot \text{min}^{-1}$ ) in response to prolonged constant work rate exercise in the GN condition.



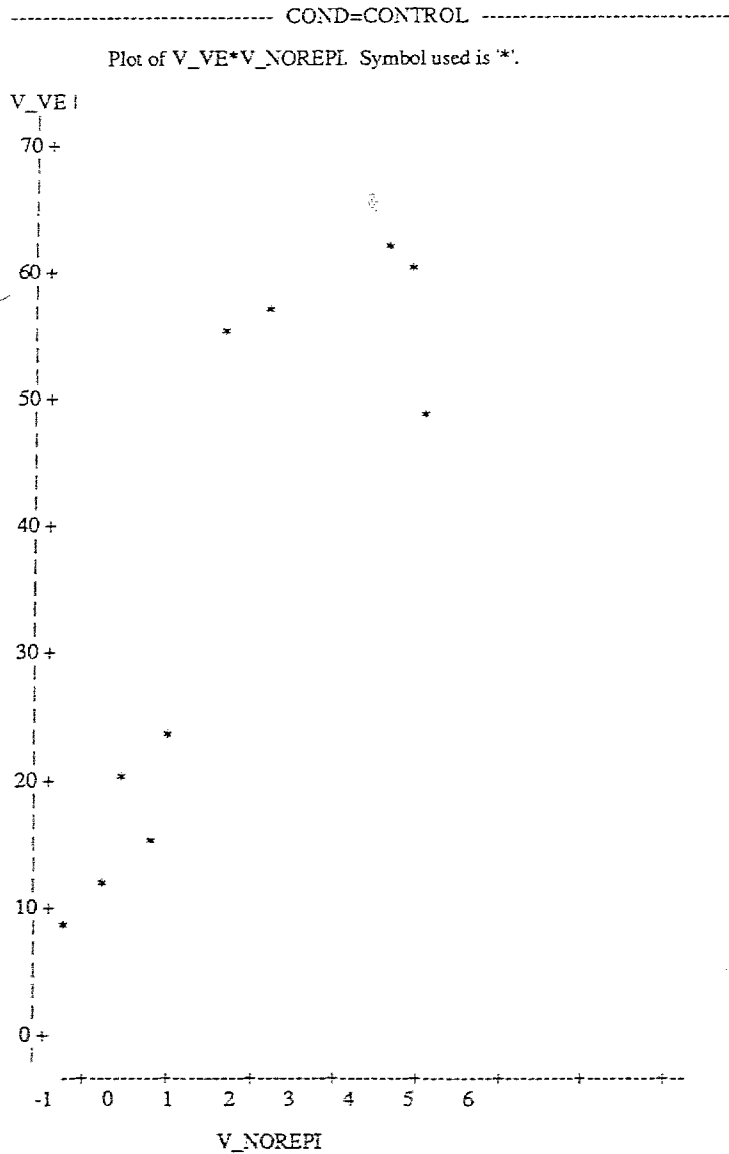
**Figure 40** Scattergram showing the individual relationship between the change in ventilation ( $\text{l}\cdot\text{min}^{-1}$ ) and the change in potassium concentration (mM) in response to prolonged constant work rate exercise in the GN condition.



**Figure 41** Scattergram showing the individual relationship between the change in ventilation ( $l \cdot \text{min}^{-1}$ ) and the change in pH in response to prolonged constant work rate exercise in the GN condition.

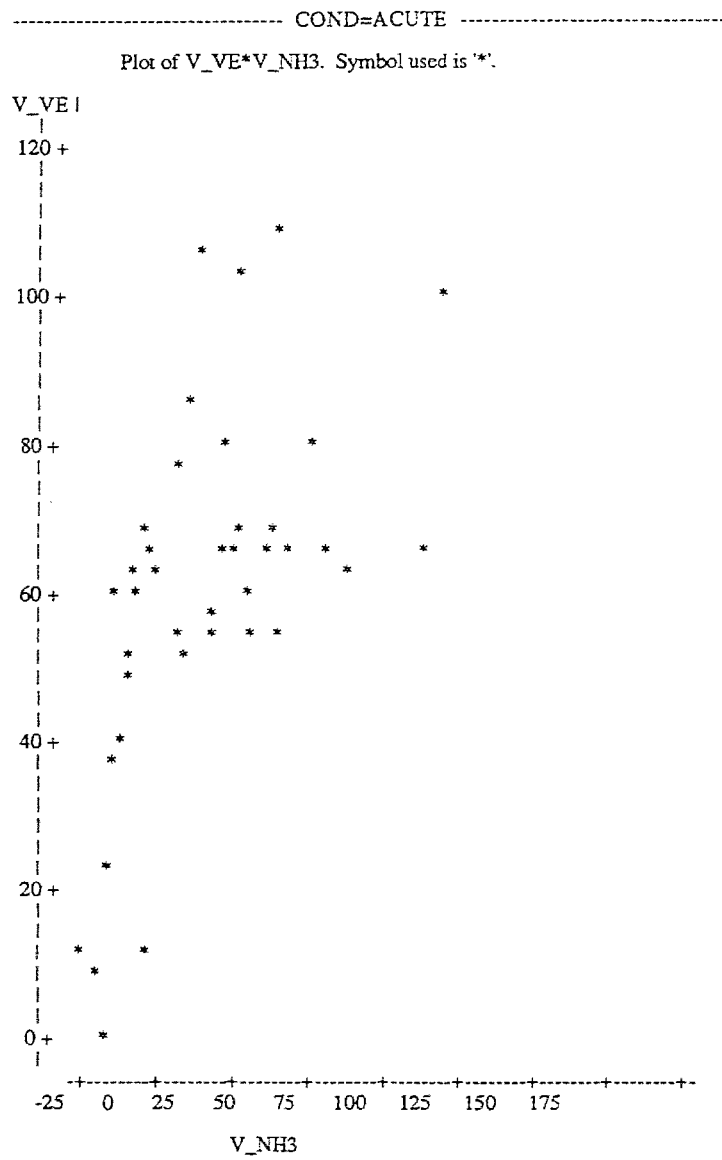


**Figure 42** Scattergram showing the individual relationship between the change in ventilation ( $\text{l}\cdot\text{min}^{-1}$ ) and the change in core temperature ( $^{\circ}\text{C}$ ) in response to prolonged constant work rate exercise in the GN condition.

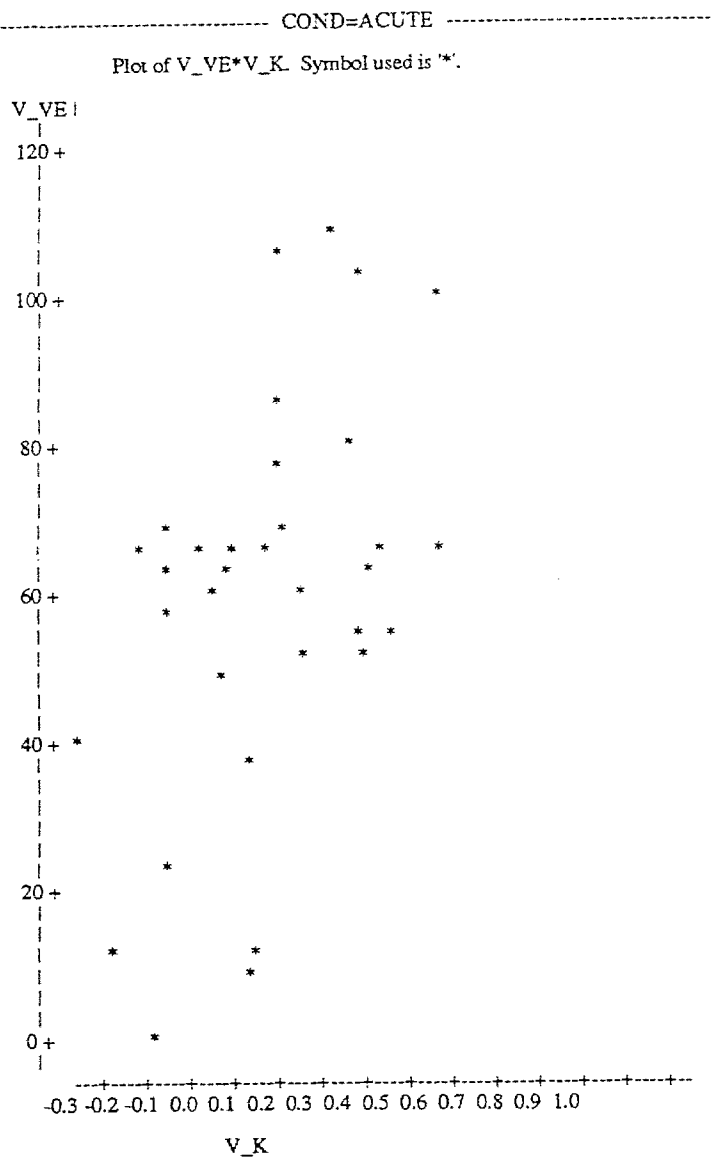


**Figure 43** Scattergram showing the individual relationship between the change in ventilation ( $\text{l}\cdot\text{min}^{-1}$ ) and the change in norepinephrine concentration (nM) in response to prolonged constant work rate exercise in the GN condition.

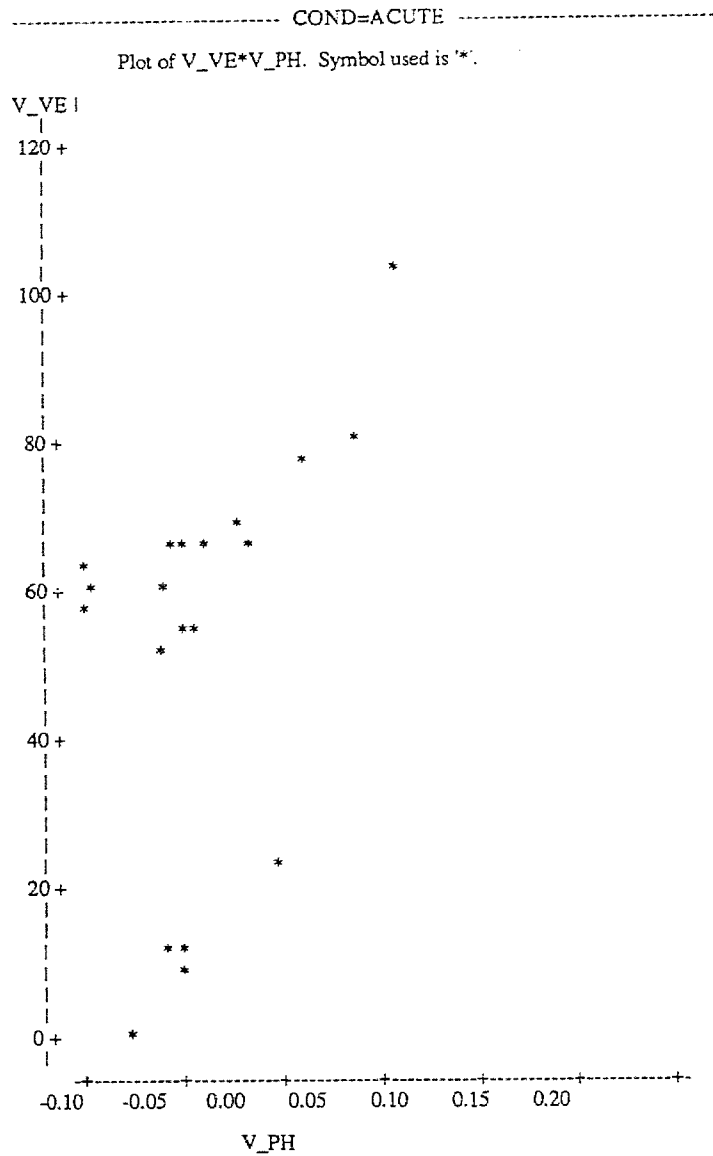




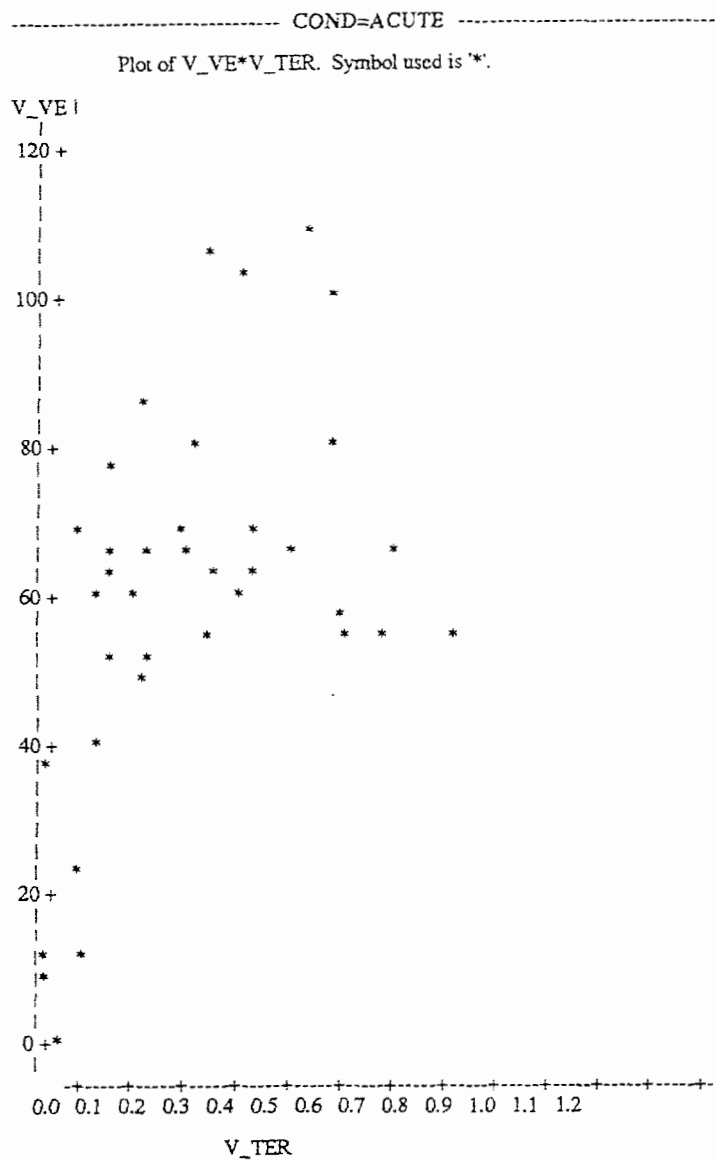
**Figure 44** Scattergram showing the individual relationship between the change in ventilation ( $l \cdot \text{min}^{-1}$ ) and the change in ammonia concentration ( $l \cdot \text{min}^{-1}$ ) in response to prolonged constant work rate exercise in the  $GD_{ACUTE}$  condition.



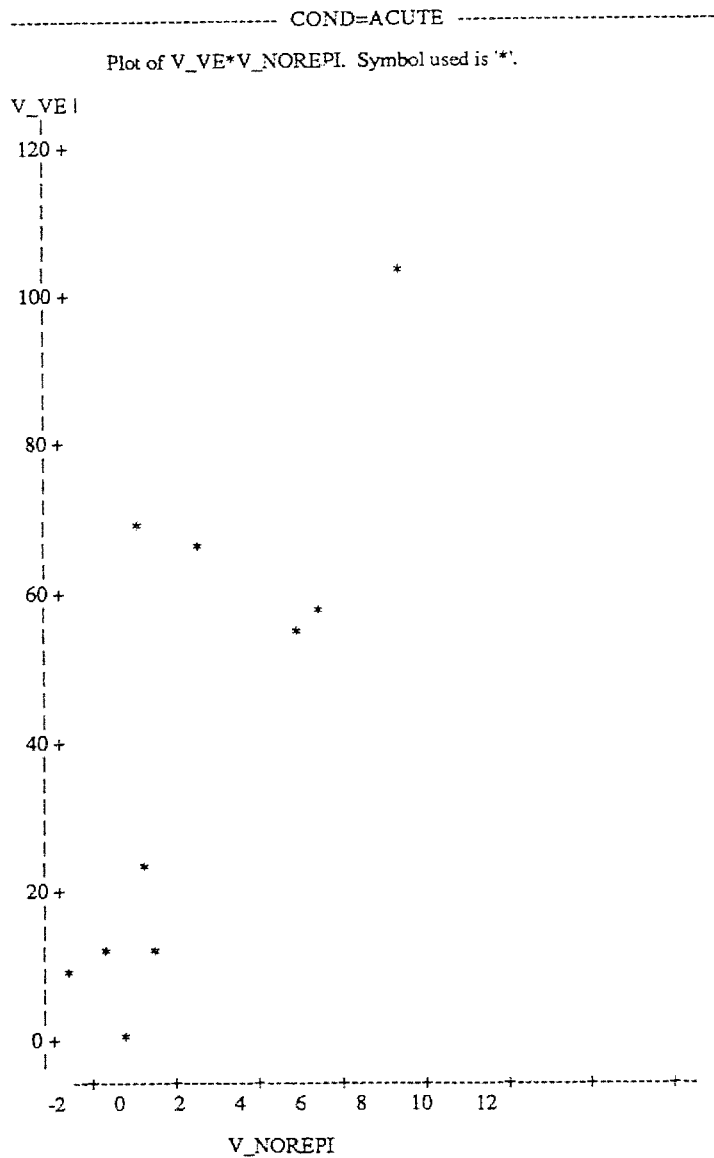
**Figure 45** Scattergram showing the individual relationship between the change in ventilation ( $l \cdot \text{min}^{-1}$ ) and the change in potassium concentration (mM) in response to prolonged constant work rate exercise in the  $GD_{ACUTE}$  condition.



**Figure 46** Scattergram showing the individual relationship between the change in ventilation ( $\text{l}\cdot\text{min}^{-1}$ ) and the change in pH in response to prolonged constant work rate exercise in the  $\text{GD}_{\text{ACUTE}}$  condition.



**Figure 47** Scattergram showing the individual relationship between the change in ventilation ( $l \cdot \text{min}^{-1}$ ) and the change in core temperature ( $^{\circ}\text{C}$ ) in response to prolonged constant work rate exercise in the  $\text{GD}_{\text{ACUTE}}$  condition.



**Figure 48** Scattergram showing the individual relationship between the change in ventilation ( $\text{l}\cdot\text{min}^{-1}$ ) and the change in norepinephrine concentration (nM) in response to prolonged constant work rate exercise in the  $\text{GD}_{\text{ACUTE}}$  condition.

## Appendix V

### Reference:

Banister, E.W., and B.J. Cameron (1990) Exercise-induced hyperammonemia:  
Peripheral and central effects.

**Int. J. Sports Med. 11(Supp. 2): S129-S142.**

# International Journal of Sports Medicine

Supported by the German Society of Sports Medicine

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## Exercise-Induced Hyperammonemia: Peripheral and Central Effects

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### Abstract

*E. W. Banister and B. J. C. Cameron, Exercise-Induced Hyperammonemia: Peripheral and Central Effects. Int J Sports Med. Vol 11, Suppl 2, pp S129-S142, 1990.*

The intent of this paper is to review the recent literature on exercise-induced hyperammonemia (EIH) and to compare the current interpretations of ammonia accumulation during exercise with the recognized clinical symptoms of progressive ammonia toxicity. In doing so, we will speculate on possible exercise-induced symptoms of CNS dysfunction which could result from elevated ammonia during intense short-duration or prolonged exercise.

Ammonia is a ubiquitous metabolic product producing multiple effects on physiological and biochemical systems. Its concentration in several body compartments is elevated during exercise, predominantly by increased activity of the purine nucleotide cycle (PNC) in skeletal muscle. Depending on the intensity and duration of exercise, muscle ammonia may be elevated to the extent that it leaks (diffuses) from muscle to blood, and thereby can be carried to other organs. The direction of movement of ammonia or the ammonium ion is dependent on concentration and pH gradients between tissues. In this manner, ammonia can also cross the blood-brain barrier (BBB), although the rate of diffusion of ammonia from blood to brain during exercise is unknown. It seems reasonable to assume that exhaustive exercise may induce a state of acute ammonia tox-

icity which, although transient and reversible relative to disease states, may be severe enough in critical regions of the CNS to affect continuing coordinated activity. Regional differences in brain ammonia content, detoxification capacity, and specific sensitivity may account for the variability of precipitating factors and latency of response in CNS-mediated dysfunction arising from an exercise stimulus, e. g., motor incoordination, ataxia, stupor.

There have been numerous suggestions that elevated ammonia is associated with, or perhaps is responsible for, exercise fatigue, although evidence for this relies extensively on temporal relationships. Fatigue may become manifest both as a peripheral organ or central nervous system phenomenon, or combination of both. Thus, we must examine the sequential or concomitant changes in ammonia concentration occurring in the periphery, the central nervous system (CNS), and the cerebrospinal fluid (CSF) induced by any effector, not only exercise, to interpret and rationalize the diverse physical, physiological, biochemical, and clinical symptoms produced by hyperammonemic states. Since more is known about elevated brain ammonia during other diverse conditions such as disease states, chemically induced convulsion, and hyperbaric hyperoxia, some of these relevant data are discussed.

### Key words

Ammonia, brain, central fatigue, peripheral fatigue, purine nucleotides

### Abbreviations

The following abbreviations are used in the text, figures, and tables.

AAA=aromatic amino acid  
AcCoA=acetyl coenzyme A  
ADP=adenosine diphosphate  
AMP=adenosine monophosphate

Asp Ac=aspartic acid  
ATA=atmospheres absolute of pressure  
ATP=adenosine triphosphate  
BBB=blood brain barrier  
BCAA=branched-chain amino acid  
BC- $\alpha$ -keto acid dehydrogenase=branched-chain alpha-keto acid dehydrogenase  
BC 2-oxo acid dehydrogenase=branched chain 2-oxo acid dehydrogenase  
CSF=cerebrospinal fluid  
CNS=central nervous system  
GAD=glutamate decarboxylase  
ECS=extracellular space



EIH=exercise-induced hyperammonemia  
 EPEN=ependyma  
 FFA=free fatty acid  
 FG=fast-twitch glycolytic muscle  
 FOG=fast-twitch oxidative glycolytic muscle  
 GABA=gamma-aminobutyric acid  
 GLN=glutamine  
 GLU=glutamate  
 5-HT=5-hydroxytryptamine  
 IMP=inosine monophosphate  
 ISOLEU=isoleucine  
 $\alpha$ -KG Ac=alpha-ketoglutaric acid  
 Lac/Pyru= ratio of lactate to pyruvate  
 LEU=leucine  
 MAO=monoamine oxidase  
 $\alpha$ -methyl-p-tyrosine=alpha-methylparatyrosine  
 NAA=neutral amino acid  
 NADH/NAD= ratio of reduced to oxidized nicotine adenine dinucleotide  
 $\text{NH}_3$ =ammonia  
 $\text{NH}_4^+$ =ammonium ion  
 NE=norepinephrine  
 OAA=oxaloacetic acid  
 OHP=oxygen at high pressure  
 PCr=phosphocreatine  
 PFK=phosphofructokinase  
 PHE=phenylalanine  
 PNC=purine nucleotide cycle  
 Pyr Ac=pyruvic acid  
 SO=slow oxidative muscle  
 Succ Ac=succinic acid  
 TRP=tryptophan  
 TYR=tyrosine  
 VAL=valine

Note: In this paper,  $\text{NH}_3$  or ammonia are also used for the sum of  $\text{NH}_3$  (ammonia) and  $\text{NH}_4^+$  (ammonium ion), recognizing that  $\text{NH}_3$  and  $\text{NH}_4^+$  are in equilibrium ( $\text{NH}_3 + \text{H}^+ \rightleftharpoons \text{NH}_4^+$ ). The  $\text{pK}_a$  of this reaction is 9.3; thus, at physiological pH, most ammonia is present as  $\text{NH}_4^+$ .

### General Ammonia Metabolism

Whatever the source or fate of metabolically produced ammonia, there always seems to be some spilled to the blood. Thus, ammonia formed in one organ may be distributed widely in the body via the circulation.

Ammonia generated in the gut (169, 176) from protein digestion and deamination of glutamine enters the portal venous circulation in the amount of several grams per day in normally active, well-nourished adults (152). Peripheral arterial concentration of ammonia is kept relatively low at rest, as shown in Table 1 (34), since the liver efficiently removes most gut-derived ammonia for excretion or recirculation as urea, creatinine, glutamine, and ammonium ion (60, 61, 62, 152).

Gut-derived nitrogen from the intestine appears in the circulation mainly as urea and glutamine, whereas labeled ammonia-nitrogen from tissue other than the gut appears in the amide group of glutamine (37, 148). The kidney releases  $\text{NH}_3$  predominantly to the urine for excretion, although

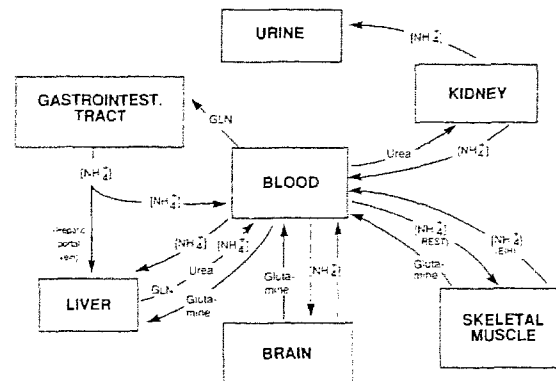


Fig. 1 Major organs of ammonia formation, utilization, and circulation either as free base  $\text{NH}_3$ , ammonia ion  $\text{NH}_4^+$ , or related nitrogenous by-products [modified from Kvamme (87), Fig.1, with permission].

Table 1 Normal concentrations of ammonia in human blood and CSF and in rat blood, CSF, and brain

	Ammonia concentration ( $\mu\text{M}$ )	Selected references
Human		
Arterial blood/plasma	22-113	42, 84, 91
Venous blood/plasma	20-25	5, 6, 24, 51, 153, 179
CSF	20-100	46, 162
Rat		
Brain	150-300	9, 32, 33, 41, 68, 70, 142, 144
Arterial blood/plasma	50-250	9, 33, 53, 70, 142, 144
Venous blood/plasma	50-80	39
CSF	100-300+	68, 70
Portal venous	350	34
Hepatic venous	40	34

(Some data are extrapolated from graphs. Tissue concentrations were interpreted as  $\mu\text{mol}\cdot\text{kg}^{-1}$  wet weight, modified from ref. 34, Table 1, with permission.)

some is also liberated to systemic blood via the renal vein (77). It may be noted that extrahepatic shunting of a hyperammonemic load to the systemic circulation is also evident in disease states of the liver, e. g., during the development of hepatic encephalopathy (4, 119). The extended life of metabolically produced ammonia is emphasized by the pathway of ammonia escaping the gut and liver to be metabolized by extrahepatic organs to glutamine. Thereafter, glutamine may again be taken up by the gut as an energy source until its remaining protein-nitrogen is excreted as urea, and its carbon skeleton as carbon dioxide and water (61, 62, 118).

To these evident intra organ exchanges must now be added the complex two-way interchange between the brain and systemic circulation. Lockwood et al. (91) have described ammonia transport into the brain (discussed later in this review). In addition, recent evidence, although is has been disputed (96), indicates that ammonia is returned to the circulation from the CSF either as glutamine (26, 79, 80, 81) or as ammonia (31, 34, 125).

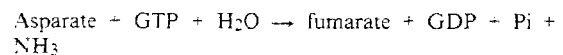


Fig. 2 Physical signs of peripheral and CNS fatigue resulting from the chronic sustained exhaustive effort of long-distance running under alien environmental conditions. Middle: at altitude, the left runner has been lapped, the middle runner who wins the race shows little physical distress, while the right runner shows fatigue and motor incoordination. Top left: at altitude, a runner suffers complete physical collapse and obvious pain. Right: the marathon runner demonstrates classical ataxia. Stupor is also reflected by the vacant facial expression.

This complex metabolism, intra organ shunting, and excretion of nitrogenous products is shown diagrammatically in Fig. 1 (87).

At the cellular level, ammonia production in different tissues is principally from:

- Deamination of glutamine catalyzed by glutaminase (38, 161):  
 $L\text{-glutamine} + H_2O \rightarrow L\text{-glutamate} + NH_3$
- The reversible oxidative deamination of glutamate catalyzed by glutamate dehydrogenase (13, 14, 154):  
 $glutamate + NAD(P)^+ + H_2O \rightarrow \alpha\text{-ketoglutarate} + NAD(P)H + H^+ + NH_3$
- Action of the PNC (principally in muscle but also in the brain and other organs) (92, 93, 136, 137, 157):  
 $AMP + H_2O \rightarrow IMP + NH_3$   
 $IMP + aspartate + GTP \rightarrow adenylosuccinate + GDP + Pi$   
 $Adenylosuccinate \rightarrow AMP + fumarate$   
 Equivalent to:



- Deamination of other amino acids  
 Transamination to an  $\alpha$ -keto acid, followed by oxidative deamination
- Oxidative deamination of monoamine neurotransmitters by MAO (63, 115) which may be an important regional source of ammonia in the brain:  
 $R\text{-CH}_2NH_2 + O_2 + H_2O \rightarrow R\text{-CHO} + NH_3 + H_2O_2$

#### Hyperammonemia of Exercise

In the exercise physiology literature, ammonia produced by exercising muscle has been associated with fatigue. Previous review of EIH (8, 105) have provided a historical perspective of the association of muscle-linked hyperammonemia to exercise-induced fatigue. Comprehensive recent reviews also clearly indicate the central role played by ammonia in the biochemistry and physiology of the brain (13, 14, 34, 39, 87). It would seem that, as a consequence of their shar-

**Table 2** Exchange of NH<sub>3</sub> across the leg and splanchnic circulation at rest and during exercise

	Rest*	Submax exercise*			Maximum**
Relative work Intensity (% V <sub>O</sub> <sub>2</sub> max)	Basal	35±2	55±3	80±3	100
Exercise time (min)	0	15	30	45	4
Arterial plasma Ammonia (μmol·l <sup>-1</sup> )	21.8±1.7	27.4±2.1	45.6±5.8	84.4±12.1	112±17
Leg exchange (μmol·min <sup>-1</sup> )	-2.4±0.5	3.6±2.5	14.4±1.9	45.7±15.3	89±21
Leg blood flow (l·min <sup>-1</sup> )	0.49±0.03	2.31±0.15	3.55±0.14	4.74±0.18	6.32±0.25
Splanchnic exchange (μmol·min <sup>-1</sup> )	-12.4±1.8	-11.4±1.1	-13.7±1.3	-14.8±3.6	n. m.
Hepatic blood flow (l·min <sup>-1</sup> )	1.27±0.12	1.01±0.06	0.72±0.06	0.40±0.07	n. m.

Data from \* ref. 42 and \*\* ref. 84, with permission. Values are reported as mean±SEM. nm=not measured. Negative sign for flux rates denotes net uptake.

ing a common circulation and a pervasive, blood-soluble, toxic metabolite, the overt features of so-called PERIPHERAL and CENTRAL FATIGUE, i. e., muscle weakness, motor incoordination, stupor, and ataxia, may be inextricably linked (Fig. 2).

During exercise a shift takes place both in the predominant source of metabolic ammonia production and also the blood supply to major organs (131). Active skeletal muscle now becomes a major source of ammonia (5, 6, 24, 35, 38, 100) by deamination of AMP to IMP in a cyclical process called the purine nucleotide cycle (PNC) (92). This cycle is also active in the brain (136, 137), although a change in its activity during exercise has yet to be investigated. There has been some argument about whether the kinetic characteristics of the enzymes catalyzing each step of the PNC in muscle are altered when physiological conditions deviate from those at rest. Meyer and Terjung have suggested that the deamination step of the PNC occurs preferentially during exercise, while the reamination of IMP to AMP proceeds more favorably during recovery (102). Flow of AMP through the PNC may be affected by other metabolic reactions since AMP may also be degraded by dephosphorylation to adenosine. The potential for ammonia production from AMP in any particular fiber type depends on the ratio of the enzymes 5' nucleotidase (AMP phosphatase) to AMP deaminase, which varies as a function of the oxidative capacity of striated muscle (21, 100, 158). Tissues with a high potential for ammonia production, as estimated by high activity of AMP deaminase, appear to have a relatively low potential for adenosine production. The relative distribution of these enzymes in striated muscle is:

(1) AMP deaminase:  
FG > FOG > SO > heart

(2) 5' nucleotidase (AMP phosphatase):  
heart > SO > FOG > FG

Other potential contributors to EIH include:

(i) deamination of amino acids, possibly during long-en-

duration performance which stimulates protein uptake and amino acid catabolism in skeletal muscle (91), particularly branched-chain amino acids, (ii) decrease in renal blood flow during exercise, which could reduce renal uptake and excretion of ammonia (131), and (iii) reduced liver blood flow and extra hepatic shunting of ammonia to the systemic circulation (42, 44, 84, 131).

It is evident, therefore, that hyperammonemia accompanying exercise in humans arises from several sources. During EIH the ammonia load represented by the above reactions may be temporarily held in the circulation before uptake by other organs for further catabolism and excretion, or it may remain permanently buffered in the blood by incorporation into other nitrogenous products.

Factors influencing the rate of ammonia production by skeletal muscle during exercise include relative muscle fiber composition (38, 170), exercise intensity, and exercise duration (5, 6, 24, 55, 175), which determine the demand for ATP formation as well as the extent of motor unit/muscle fiber recruitment (64).

Previous suggestions that production of ammonia may stimulate glycolysis (94, 150) and therefore lactate production have been challenged (55), as has been the role that ammonia may play in buffering hydrogen ion during exercise (84). Recent investigations have demonstrated clearly that EIH is not an obligatory adjunct to exercise-induced lactacidosis (55). The environmental PO<sub>2</sub> level appears to have paradoxical effects on EIH. Hyperoxia results in an elevated muscle and plasma ammonia (55) but less elevation of lactate (10, 55, 73, 155, 171, 177), whereas hypoxic acclimation reduces EIH, at least during submaximum exercise (179). These apparently contradictory results also contrast with initial experimental evidence that ammonia is produced predominantly from fast-twitch muscle, particularly during intense (anaerobic) exercise (38, 100, 101, 170).

### Fate of Ammonia in EIH

Because of increased muscle  $\text{NH}_3$  production during exercise, there is a shift from the net uptake of ammonia in skeletal muscle observed at rest to a large net efflux into the circulation during exercise in humans, which increases in magnitude as exercise intensity increases (Table 2) (42, 84). The important function of skeletal muscle in removing circulating ammonia at rest (72, 91) may therefore be reduced or reversed (42, 84), although nonactive muscle may still provide a venue for the uptake of ammonia from the blood.

The mounting hyperammonemic load faced by the body during exercise is evident in a rising blood ammonia concentration, although the imbalance between ammonia production and removal may be intermly contained by the blood and exercise continued for a considerable period. The liver, which normally regulates blood ammonia, appears not to increase its rate of ammonia extraction ( $\approx 12\text{--}15 \mu\text{mol}\cdot\text{min}^{-1}$ ) during exercise, although the arteriovenous difference for  $\text{NH}_3$  across the liver must increase since blood flow to the liver decreases during exercise (Table 2) (42, 84). Because the circulating ammonia concentration is increased, every area of the body is now exposed to a potential hyperammonemia.

What mechanisms restrain exercise hyperammonemia? The current literature attributes exercise-induced ammonia flux principally to the action of MUSCLE (production), BLOOD (circulation), and LIVER (detoxification). This appears inadequate either during submaximal or intense exercise. Thus, we view the blood as an important storage compartment with a role in temporarily accommodating and redistributing an acute or chronic ammonia load in plasma (33, 60, 61, 118). If existing modes of blood detoxification become limited during exercise either by reason of decreased blood flow to vital organs or by saturation of their detoxifying power, we speculate that little protection would then remain to the brain against a chronic and increasing ammonia load. It is evident that blood ammonia is elevated during exercise, and it is equally well documented that ammonia crosses the blood-brain barrier (from blood to brain) under the influence of both concentration and pH gradients. Lockwood et al. (91) suggest that in normal subjects (at rest)  $\text{NH}_3$  is taken up from blood by liver, skeletal muscle, bladder, and brain, and that within the brain itself, ammonia uptake is greatest in grey matter, i. e., cell bodies. Currently little information is available on the accessibility of circulating blood ammonia to the brain during exercise in normal healthy individuals. We have found only one paper which reports elevated brain ammonia in rats during EIH (113). While the absolute values reported here in both blood and brain seem somewhat high, the pattern of their elevation is consistent with the observed elevation of blood and brain ammonia in other conditions (encephalopathy, hyperoxia, etc.; 52, 76, 133, 142, 144).

### Pattern of Ammonia Accumulation in Organs

Exercise is one of a variety of stimuli effecting a transient or chronic hyperammonemia. The common pattern of clinical symptoms which signifies developing toxicity induced by such disparate conditions as chemical poisoning, electric shock disease, or hyperoxia seems to proceed from pe-

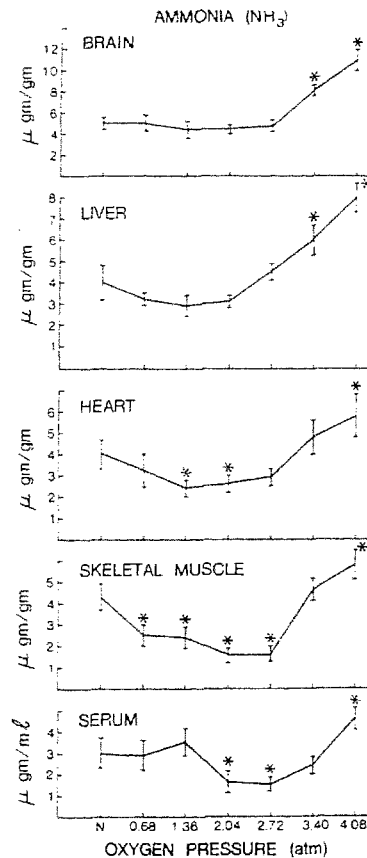


Fig. 3 The progressive hierarchical elevation of ammonia concentration in various rat tissues due to a hyperoxic stimulus in resting animals up to an oxygen pressure producing convulsion [from Singh and Banister (144), Fig. 1, with permission].

ripheral involvement to central (CNS) dysfunction (34, 43, 117, 120, 151). It seems unlikely, therefore, that the accompanying pattern of organ hyperammonemia differs markedly in response to the different stimuli. A hierarchical picture of exercise-induced ammonia accumulation in major organs may perhaps be inferred from experiments in which hyperammonemia has been induced by a different stimulus and specific tissue ammonia measured. Fig. 3 (144) shows the pattern of developing hyperammonemia at rest in animals in response to an incremental hyperbaric oxygen stimulus, sufficient eventually to produce convulsions, a condition reversible when the stimulus is removed. During such exposure, the liver is the first organ to show a sustained progressive ammonia elevation, followed by the heart, skeletal muscle, serum, and brain. Convulsive activity usually accompanied a brain ammonia concentration of  $0.90\text{--}1.10 \mu\text{mol}\cdot\text{g}^{-1}$ . Confirmation of whether a similar temporal order of developing tissue hyperammonemia exists during an incremental exercise stimulus to exhaustion awaits development of adequate experimental techniques to determine brain ammonia flux during exercise in humans. However, the rise of blood ammonia above some critical level with other stimuli seems to signal the onset of CNS symptoms severe enough to curtail

coordinated activity in animals and man. Thus, acute ammonia loading of rats with a preexisting low-grade hyperammonemia induces acute physical (106) and electrophysiological (22, 124) signs of CNS disruption.

#### Fate of Ammonia in the Brain

Ammonia is an important metabolite in endogenous brain metabolism. Under resting conditions the ammonia content of the brain is maintained at a relatively low concentration (Table 1) (34). Any substantial extraneous influx of  $\text{NH}_3$  across the BBB may seriously unbalance its equilibrium. It is now acknowledged that ammonia has access to the brain from the blood predominantly as free base  $\text{NH}_3$ , but also as the  $\text{NH}_4^+$  ion (31, 32, 34, 125). Its movement is directly dependent on concentration and pH gradients (32, 33, 91, 147, 167). When ammonia is presented to brain tissue in a large single dose, at a rapid rate, or in conjunction with an already established elevated condition, existing endogenous detoxification mechanisms are unable to contain the increased ammonia load, and the brain ammonia concentration rises rapidly (53, 67). Over a period of continuing hyperammonemic challenge, the toxic central effect of ammonia becomes magnified and manifest via the CNS causing widespread rather than local dysfunction, as noted in the previous section.

The glutamate-glutamine system (14, 15) is a principal detoxification pathway for ammonia in the brain. Evidence for this is that following continuous common carotid infusion of nitrogen label from [ $^{15}\text{N}$ ] ammonia, the label rapidly appears principally in the amino group of glutamate and in both glutamine nitrogens (37, 148). Labeled carbon appears in glutamine within 1 min of a large L-[ $^{14}\text{C}$ ] glutamate infusion intracisternally (18), indicating a rapid turnover in a small active glutamate pool in the astrocyte rather than in a whole brain glutamate compartment (Fig. 4). Glutamine appears to act as a principal intermediary of two-way ammonia-nitrogen exchange across the BBB (1, 2, 39, 49, 53) in the regulation of brain ammonia. A direct loss, < 3% of the total brain  $\text{NH}_3$  free base, occurs at rest (31, 125). The extent of this loss or gain during exercise, however, is unknown.

In associated reactions, glutamate may also undergo oxidative decarboxylation by glutamate decarboxylase (GAD) to form GABA (65, 66, 130). Glutamate and GABA, respectively, have defined excitatory and inhibitory actions as neurotransmitters, while glutamine has no known neurotransmitter action (86).

Regional differences in the capacity for ammonia removal (buffering) have been demonstrated for brain tissue. Butterworth et al. (25) have suggested that the cerebral cortex (CC) has only a limited capacity to remove blood-borne ammonia by the formation of glutamine compared with the brainstem. This is due to a moderate decrease of glutamine synthetase (GS) activity in the CC accompanying hyperammonemia. Thus, ammonia concentration may become regionally elevated in a manner which could be disruptive to coordinated activity. As an example, inhibitory postsynaptic transmission (IPSPs) in the brain is directly and negatively affected by hyperammonemia (76, 95, 121, 123). The resultant disinhibition in regulatory control is almost certainly associated with

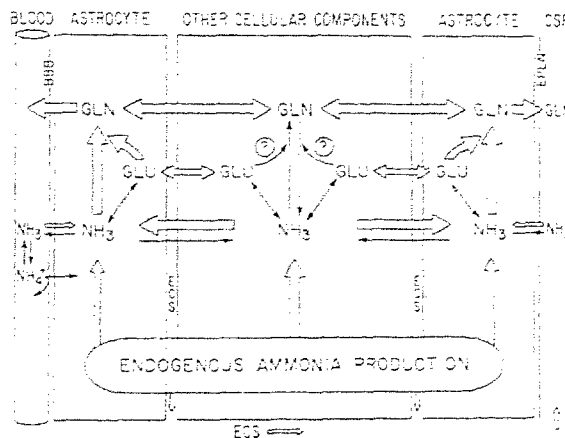


Fig. 4 Compartmentation of ammonia metabolism in rat brain. Arrow thickness indicates the relative importance of various pathways [from Cooper and Plum (34), Fig. 1, with permission].

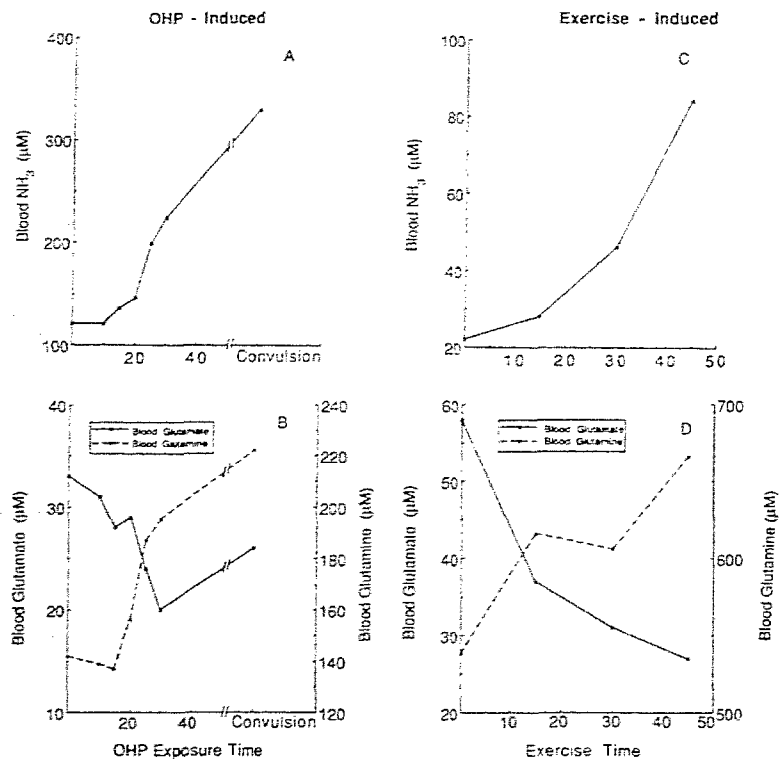
developing clinical symptoms of ammonia toxicity in humans. Neurological symptoms ascribed to ammonia toxicity include abnormal locomotor behavior (74), altered sleep pattern (12), and modification of neuromuscular coordination (52).

#### Ammonia from Protein Catabolism During Exercise - Its Influence on CNS Toxicity

Exercise exerts several important effects on protein metabolism which may be relevant to CNS toxicity. Firstly, it stimulates catabolism of amino acids in muscle (principally BCAAs) and contributes to elevated blood ammonia (90); secondly, the hyperammonemia accompanying exercise increases the permeability of the BBB to NAA relative to other amino acids (26, 48, 97, 138); thirdly, the circulating BCAA fraction of the NAA group is reduced relative to the AAA fraction, which are neurotransmitter precursors, probably due to enhanced BCAA uptake by active skeletal muscle (2, 20, 28, 113). Thus, the AAAs (Phe, Tyr, and TRP) are positioned more favorably for uptake across the BBB (79, 80, 81).

Skeletal muscle has a well-developed capacity for amino acid catabolism, particularly the BCAAs (LEU, ISOLEU, VAL) (90). The necessary enzymes for degradation of BCAAs are found principally in skeletal muscle (103). Exercise also increases the activity of a principal enzyme (BC 2-oxo acid dehydrogenase) which continues the degradation of BCAAs, after an initial transamination, to glucogenic and ketogenic residues (165).

Enhanced uptake of the neurotransmitter precursors (PHE, TYR, and TRP) may contribute to a neurotransmitter imbalance within the CNS (3, 79, 80, 81). Romanowski and Grabiec (128) were first to report an exercise-induced increase in brain serotonin (for which TRP is the precursor) and were also probably the first to speculate on its potential role in mediating CENTRAL FATIGUE. Several subsequent studies have supported this theory, reporting an exercise-induced decrease in the ratio of BCAA/AAA in blood, or an increase in the brain uptake of AAA and brain



**Fig. 5** Corresponding changes in blood ammonia, glutamate, and glutamine induced by high pressure oxygen (OHP) leading to convulsion (5A, 5B) and by exercise-induced hyperammonemia (5C, 5D). Quantitative differences may be accounted for by species differences [data from Singh and Banister (142) (Fig. 5A, 5B) and Eriksson et al. (42) (Fig. 5C, 5D), with permission].

serotonin concentration (2, 20, 28, 29, 113). Elevated brain serotonin resulting from exercise could trigger such fatigue-related symptoms as lethargy, appetite suppression, and sleep disorders (23, 126). A recent report, however, seems to challenge these ideas, indicating that TRP ingestion prior to exercise, which would potentially increase brain serotonin synthesis, enhanced treadmill running endurance by almost 50% (140). Increased serotonin concentration was postulated to decrease sensitivity to pain, thus allowing intense exercise to continue significantly longer.

Chronic elevation of brain NE and serotonin (23), both of which are synthesized from AAA precursors, has also been reported in response to endurance training. A potentially negative aspect of the above exercise-induced AAA response is that endogenous brain ammonia could be significantly increased by enhanced deamination of brain catecholamines and serotonin. Augmented catecholamine turnover with no change in the whole brain catecholamine pool size has been clearly demonstrated in rats during OHP exposure leading to brain hyperammonemia, using an  $\alpha$ -methyl-tyrosine block of catecholamine synthesis (9).

In spite of the above negative effects, the hyperammonemia of exercise may also induce a balancing set of reactions important for brain homeostasis which partially restores the glutamate carbon and nitrogen pool. Active CO<sub>2</sub> fixation in the astrocyte (cells in the brain which bridge between capillaries and neurons) is stimulated by ammonia (19, 164) and replenishes the carbon skeleton for GLU and GLN synthesis. Glutamate-nitrogen may also be replenished in the astrocyte by amino acid uptake from the blood, principally from BCAAs (45), in addition to active uptake of GLU, ASP,

and GABA (65, 66). By contributing to replenishment of the astrocyte GLU pool (32), BCAA uptake by the brain, which continues in spite of a reduced plasma BCAA/AAA ratio, is viewed by some investigators as occupying a pivotal role in the glutamate-glutamine cycle of the brain (34). During exercise the absolute plasma concentration of BCAAs actually rises, and is two to three times higher than AAAs (2, 20, 113). Although the ratio of BCAA/AAA declines steadily throughout endurance exercise, it seems to remain above 2.0 (20, 113). The importance of continued BCAA uptake by the brain is illustrated by the reported effect that restoration of the "normal" brain BCAA/AAA ratio (by BCAA infusion) has upon reducing ammonia-induced toxicity in hyperammonemic animals (48) and possibly in humans with liver disease (127, 129).

Although disputed (96, 97), it has been proposed that the uptake of BCAAs relative to AAAs is facilitated by a concomitant GLN efflux of ammonia-nitrogen from the brain (26, 79, 80, 81, 127). Glutamine is reportedly uniquely synthesized *in situ* in brain microvessel epithelium and astrocytes by enhanced glutamine synthetase activity (26, 50, 82, 108, 112). Ammonia-induced disruption of the fine structure of the astrocyte-microvessel anatomical site of the BBB (36, 109, 111, 163) may also increase its permeability to NAAs, as noted above.

#### Comparison of the Ammonia - Glutamine System in Blood and Brain: An Extrapolation to Exercise

Evidence for a developing ammonia toxicity in the brain directly attributable or secondary to EIH is singularly lacking in the literature. Only one paper seems to have

**Table 3** Concentration of ammonia, glutamate, and glutamine in blood and brain produced by exercise, in disease states, or at the onset of coma or convulsion by other toxic stimuli. Original sources are indicated by reference numbers in the table

	NH <sub>3</sub>	Blood GLU ( $\mu$ M)	GLN	NH <sub>3</sub>	Brain GLU ( $\mu$ mol·g <sup>-1</sup> )	GLN	Selected references
<b>Human</b>							
Sub max. 80% max	84	27	666				42
Exh. ex. 97%	210	163	524				84
Exh. ex. 100%	240						6
Exh. ex. (run)	130		700				5
Exh. ex. (cycle)	90						35
Exh. ex. (handgrip)	174						145
Pathological states (with neurological symptoms)	62-1490						76
Liver disease	62-264						91
<b>Rat</b>							
Ex.	530			2.94			113
Exh. ex.	350						104
Exh. ex.	540						100
Ex. 2-h run		147	594				28
NH <sub>4</sub> C1 infusion				0.44	2.0	5.57	159
				1.00		5.00	159
OHP (convulsion)	452	32	256	1.13	5.1	3.82	7, 9, 142, 143, 144
CO <sub>2</sub> breathing		61	75.4				75
PCA	400			Cortex: 0.5		15	25
	400			Brainstem: 0.4		6.5	25
PCA (coma)	1500			Cortex: 4.5		15	25
	1500			Brainstem: 3.0		14.5	25
Lindane (convulsion)				1.25		6.25	114
Telkodrin				2.5		7.0	59

Some data are extrapolated from graphs. PCA=portacaval anastomosis; Ex.=exercise; Exh. ex.=exhaustive exercise.

addressed this topic, almost incidentally, during the study of exercise-induced stimulation of neutral amino acid transport into the brain (113). It is evident, however, that OHP exposure of the rat produces a corresponding pattern of elevation in blood ammonia and glutamine, and a concomitant reduction in blood glutamate as has also been observed during intense fatiguing exercise in humans (Fig. 5) (42, 142).

Several papers have described similar changes in these metabolites both in blood (human and animal) and brain (animal) during the course of developing hyperammonemia induced by different stimuli, including exercise (Table 3). Some data could not be included as they were reported as  $\Delta$ NH<sub>3</sub>, not as absolute values (e. g., 38). This is in spite of species differences and variability of analytical techniques introduced in the various reports (see table for cited references). The similarity in order of magnitude of the final ammonia concentration *in extremis*, i. e., either at exhaustion or convulsion, produced by exercise or a variety of other toxic stimuli in blood and brain, respectively, is compelling. It may indicate that an upper limit of tolerable organ hyperammonemia exists in the whole brain or in some critical brain compartment, above which signs of CNS dysfunction become apparent.

Fig. 6 shows that the pattern of change of ammonia, glutamate, and glutamine concentration is similar in both blood and brain during the course of animal exposure to OHP at 5.5 ATA leading to convulsion. GABA and glutamate decrease while concomitantly brain ammonia and glutamine increase (142).

### Metabolic Effects of Ammonia

Details of the metabolic effects of ammonia have been reviewed previously (34, 87, 105). A summary is presented in Table 4. It seems the multiple secondary effects of exercise hyperammonemia may be traced first to an energy deficit in the periphery enhancing ammonia production by the PNC and BCAA catabolism which leads more importantly and finally to a depletion of ATP in critical regions of the brain.

The reported effect of ammonia on specific enzyme-mediated reactions and associated metabolic pathways suggests that ammonia may alter the rate of energy production and subsequent availability of ATP. An ammonia concentration of 1-3  $\mu$ mol·g<sup>-1</sup> in the brain depletes ATP and elevates ADP and AMP, particularly in the brainstem (33, 53, 67). Brain ammonia attains these values in several hyperammonemic conditions (Table 3).

The reduction in oxidative metabolism through the Krebs' cycle and electron transport chain may not be matched by increased glycolysis induced by hyperammonemia, although conflicting observations limit precise interpretation of the role played by ammonia in intermediary metabolism.

Theoretically, the diversion of glucose carbon to glutamine synthesis through CO<sub>2</sub> fixation induced by hyperammonemia in the major detoxification process in the brain may also represent a loss of 28 of 38 equivalents of ATP potentially available from glucose oxidation (30). CO<sub>2</sub> fixa-

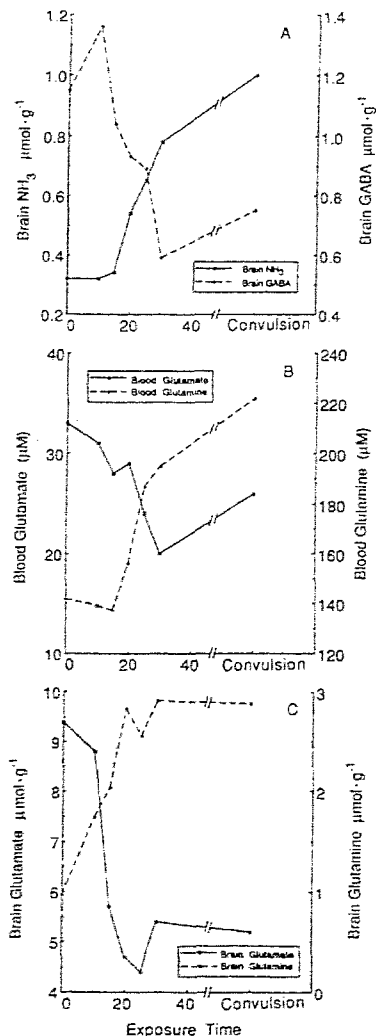


Fig. 6 Changes in blood and brain metabolites observed during DHP exposure leading to convulsions in rats. Glutamine was measured as combined glutamine and asparagine [from Singh and Banister (142), with permission].

tion, which replenishes the brain's carbon pool, is also an energy-requiring process (19, 116, 134). Carbon drain on the BBB astrocyte pool may be replenished in the astrocyte by the anaplerotic reactions described above, by neuronally derived amino acids, or NAA uptake as described earlier.

Indirect evidence supporting such a simplifying concept of energy depletion stems from prominent astrocytic changes induced by hyperammonemia, including enlargement and mitochondrial proliferation (27, 56, 57, 109, 180). Significant changes in neuronal astrocyte fine structure may reflect the intense metabolic activity needed to sustain glutamine synthesis and brain ammonia homeostasis. This may be analogous to the fine structure disruption observed in the periphery leading to proliferation of skeletal and cardiac

Table 4 Mechanisms of hyperammonemic disruption of biochemical pathways and energy metabolism. Original references are cited. ↑ indicates an increase in activity, ↓ indicates a decrease in activity, — indicates no change in activity, ↓ ? indicates a possible inhibition or depletion

Process or Reaction	Action	Reference
Adenylate cyclase (rat brain, liver & fat)	↑	107, 174
Adenylate cyclase (liver & fat)	↑	174
Glutamate decarboxylase	↑	132, 149
Glutamate dehydrogenase	↑	110, 133
Isocitrate dehydrogenase	↑	18, 83
MAO (brain)	↑	132, 148
Na-K-ATPase (brain)	↑	132, 149
PFK	↑	94, 150
Pyruvate carboxylation	↑	99
Tissue ATP	↓ ?	47, 69
BBB permeability to NAA, BCAA	↑	79, 80, 81, 97
Blood glucose, lactate, FFA, ketone bodies	↑	17, 22, 122, 141, 160
Carbamoyl phosphate synthesis (liver)	↑	146
Cerebral respiration	↑	99, 166
Electron transport chain	↓ ?	71
Energy charge ratio	—	69
Glycogen stores (skeletal muscle, heart, liver, brain)	↓	122, 174
Glycolysis	↑	99
Lac/Pyr, NADH/NAD ratios	↑	53, 70, 71
Malate-aspartate shuttle	↓	34, 70
PCr (brain)	↓	69, 98, 135
Protein synthesis (brain and liver)	↓	40, 139, 168

muscle mitochondria following their initial disruption in response to exhaustive exercise (11, 54, 58, 85, 89).

#### Integration of the EIH Effects in the Periphery and CNS

Fig. 7 summarizes the overall ammonia flux and interorgan relationships proposed to result from EIH.

Ammonia arises directly from skeletal muscle activity under exercise stress. Peripheral fatigue may be influenced by the *in situ* production of ammonia in skeletal muscle and its stimulating, but perhaps wasteful effect upon glycolytic flux, local lactic acid production, and substrate depletion. Proposed causal relationships between ammonia, lactate, and fatigue are disputed, however, and remain equivocal (78, 156, 172). It is certain, however, that muscle activity during exercise contributes in a significant manner to hyperammonemia, and that the blood compartment absorbs and distributes an increasing ammonia load to other metabolic sites including the liver and brain. During sustained, extremely exhausting exercise, the detoxification capacity of peripheral organs may become saturated and blood NH<sub>3</sub> rises. The brain thus becomes exposed to the toxicity of excess ammonia.

Endogenous sources of brain ammonia include (i) neurotransmitter deamination, (ii) oxidative deamination of GLN and GLU in nerve endings and astrocytes, respectively, and (iii) the brain PNC, some or all of which may be stimulated by exercise.





(Fig. 2). The onset of heat stroke for example is heralded by conflicting CNS-associated symptoms, e. g., a bounding or thready pulse, by aggression or apathy, by a dry red skin, or by profuse sweating. Overall there is loss of motor coordination and finally stupor and coma (178). The observed symptoms may be first interpreted as indicative of PERIPHERAL FATIGUE in which there is no accompanying loss of coordination, or lucidity of thought, or behavior, but only developing muscle weakness and an awareness of strained breathing, heart sounds, sweating, and an unwillingness to continue. Under more strenuous and alien environmental conditions, the toxic CNS effects of serious hyperammonemia become increasingly obvious so that *in extremis* CENTRAL FATIGUE is dominant in which motor control, coherent thought, and even consciousness are lost.

Acknowledgements by funds from the natural Science and Engineering.

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